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AN APPARATUS FOR THE GROWTH OF FUNGI ON MEDIA OF CONSTANT COMPOSITION[1]

T. C. LOUGHHEED[2]

Abstract

An apparatus is described for growing fungi on media of defined and constant composition. The fungus grows as a surface mat on a porous, inert membrane supported on the surface of a liquid medium. A continuous flow of the medium is maintained through the growth cell to ensure its constant composition.

A species of *Hirsutella*, parasitic on spruce budworm (*Choristoneura fumiferana* (Clem.)), tentatively identified as *Hirsutella gigantea* Petch, is being investigated in this laboratory. During a study of the factors influencing spore formation it has been noted that synnemata, the spore-bearing structure, are not common in cultures growing under ordinary laboratory conditions. Conidia have only been observed in cultures growing on solid media, where, unfortunately, this fungus grows rather slowly. It was also found that the normal technique of growing the organism on nutrient agar had some other disadvantages. The diffusion of nutrients into depleted areas makes it difficult to determine and impossible to control the concentration of the nutrients once growth has started. With liquid medium it is possible to avoid concentration gradients and also to control the nutrient concentrations by periodic sampling and replenishment. *H. gigantea* only slowly forms a surface mat on stationary liquid cultures and it is still not certain that spore-bearing synnemata will be produced. Therefore an apparatus has been developed for growing this fungus which combines the advantages of both liquid and solid culturing techniques.

The basic principle of the apparatus is that the fungus is supported on a thin porous membrane, which acts as an artificial surface mat, on the surface of a liquid medium. Several other considerations governed the actual design of the apparatus: (*a*) The composition of the nutrient solution must be constant and controllable. (*b*) The nutrient solution in the apparatus must be homogeneous and any changes made in the nutrient must affect the whole of the

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Contribution No. 527, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada. This work formed part of a Ph.D. thesis submitted to the University of London.

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[The previous number of Can. J. Botany (**37**, 331–492) was issued May 13, 1959.]

mycelial pad simultaneously. (*c*) The apparatus should be constructed of biologically inert materials (*d*). (*d*) The apparatus must be compact and robust enough to permit sterilization by autoclaving.

The diagram of the apparatus designed to meet these requirements is shown in Fig. 1 while the details of the growth cell are shown in Fig. 2.

Medium reservoir.—Any suitable glass vessel could be employed; in the present assembly a Winchester bottle was used. It was fitted with a cotton-plugged air vent and the medium was fed via a siphon.

Control valve.—The valve used is shown in Fig. 3. It was constructed entirely of glass except for the silicone tubing used to provide a flexible joint between the capsule and rod. The solid glass rod was selected to be a close fit in the capillary tube. The use of a magnetic control to regulate the flow rate by inserting or retracting the glass rod eliminated the usually troublesome packing gland or seal.

Growth cell.—The growth cell was constructed by Q. V. F. Ltd., Stoke-on-Trent, Staffs., England, using their 3-in. I. D. industrial buttress joints and clamps. These buttress joints provide a water-tight seal if properly greased, even when clamped on the membrane. The raised interior extension of the

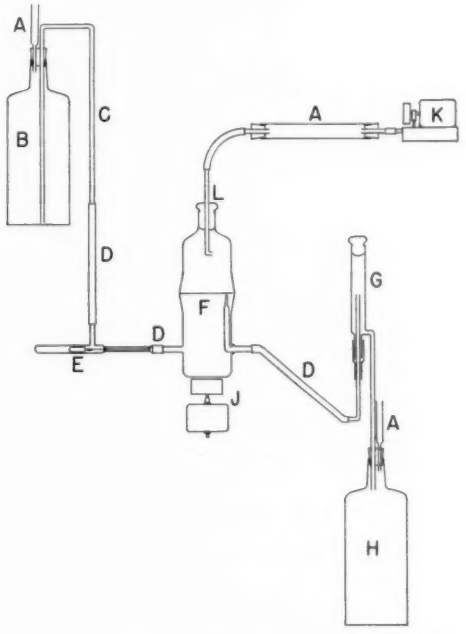

FIG. 1. The general arrangement of the apparatus. A, cotton wool air filter; B, medium reservoir; C, siphon; D, silicone tubing; E, valve; F, growth cell; G, waste overflow regulator; H, waste reservoir; J, magnetic stirrer; K, air pump; L, aeration tube.

outlet ensured that the medium flowed into the waste reservoir only after passing close to the membrane. The circular cross section of the cell together with continuous stirring assured the maintenance of uniform conditions throughout the medium. The size of the magnetic stirring bar was chosen so that, when the stirring had reached a uniform state, the medium at the surface of the membrane was just slowly circulating. The teflon sheet prevented abrasion of the glass cell by the stirrer during the long periods of stirring. The cell was supported in a stand as shown in Fig. 2. Four adaptors, which screwed into holes drilled in the lower backing flange, fitted into holes in the upper ends of the supporting posts. They were held securely in place by transverse pins. The supporting posts were fastened to a heavy cast iron base to give good stability to the assembly. A clamp for holding

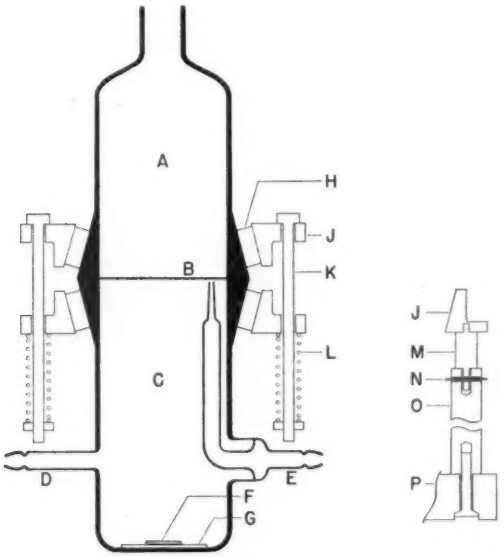

FIG. 2. A cross-sectional drawing of the growth cell. The insert shows the details of a post of the supporting stand. A, upper cell; B, membrane; C, lower cell; D, medium inlet; E, medium outlet; F, magnetic stirring bar; G, teflon sheet; H, asbestos insert; J, backing flange; K, clamping bolt; L, spring; M, adaptor; N, locking pin; O, supporting post; P, base.

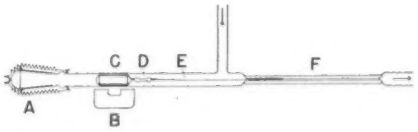

FIG. 3. Details of the flow regulator valve. A, ground glass stopper; B, magnet; C, iron rod in sealed glass capsule; D, flexible joint (silicone tubing); E, solid glass rod; F, capillary tube.

the magnetic stirrer motor was also attached to the base. The membrane, which was selected after trials of a number of materials, was glass fiber filter paper (supplied by H. Reeve Angel and Co.). This paper is not robust, so it was supported on a piece of thin, woven nylon material.

Overflow regulator.—The overflow regulator was constructed of glass and silicone tubing. The height of the adjustable tube was set so that the medium was just in contact with the whole lower surface of the membrane but air was not sucked through the membrane.

Aeration unit.—The aeration tube passed through the cotton plug in the neck of the upper cell. This tube was made with several sharp projections on the outer wall to prevent the plug from slipping along the tube. The air was sterilized by passage through a tube (1.5×25 cm) packed with cotton wool. A small air pump (Type MKI, Charles Austin Ltd., West Malling) provided sufficient pressure. The air supply was used intermittently, mainly to prevent excessive accumulation of condensed water on the walls of the upper cell.

Connections.—All flexible connections were made with translucent silicone tubing quality QS 168 as manufactured by H. D. Symons & Co. Ltd., Kingston Hill, Surrey.

The apparatus was sterilized in four units as follows.

Unit 1, the medium reservoir and control valve.—The reservoir was filled with medium to approximately $\frac{3}{4}$ of its total volume, the rubber stopper and siphon inserted and wired into position. The valve was connected to the siphon and the open end covered with a close-fitting glass vial. The assembly was autoclaved for 15 minutes at 15 lb pressure, the standard conditions used for all the units.

Unit 2, the growth cell in its stand.—The lower cell, resting in its backing flange and asbestos insert, was placed in position on the stand. The teflon sheet and magnetic stirring bar were placed in the bottom of the cell. Silicone stopcock grease was applied liberally to the rim of the cell and a piece of thin nylon material was placed over the open end of the cell and pulled taut. A circle of glass fiber filter paper, $3\frac{1}{2}$ in. in diameter, was placed on the nylon support. The upper cell, also well greased, and with the backing flange and insert in place, was lowered into position on the lower cell. The bolts were inserted, the springs placed over the bolts, and the nuts tightened up in rotation using only finger pressure. The top opening of the cell was plugged and the side arms were covered with vials before autoclaving.

Unit 3, the overflow regulator and waste reservoir.—The two pieces and the connecting tube were assembled, the end of the tube covered with a vial, and the unit autoclaved.

Unit 4, the aeration tube and air sterilizer.—The aeration tube was wrapped with cotton wool to form a plug and was inserted in a 250-ml Erlenmeyer flask whose neck diameter was the same as that of the growth cell. The sterilizer tube was attached, wrapped in brown paper, and the unit autoclaved.

Following autoclaving and cooling the units were assembled. A clamp was placed on the tube connecting the medium reservoir and valve before the valve was connected to the inlet of the growth cell. The overflow regulator was then connected to the outlet of the growth cell. Since the siphon and valve invariably filled with medium during autoclaving, medium flowed into and filled the cell when the clamp was opened. The magnetic stirrer was clamped into place below the cell and stirring started. The cell was inoculated by pouring 20–25 ml of dispersed mycelium on the upper surface of the membrane. The excess liquid soon drained through the membrane into the lower cell. The plain cotton plug was replaced by the aeration tube and plug after inoculation. The valve was adjusted to give a flow rate of approximately 3 ml/hour. A bottle containing 2 liters of medium lasted about four weeks and, when exhausted, it was replaced by a fresh bottle of sterile medium.

In one experiment conducted with this apparatus, *H. gigantea* was grown using glucose (0.2%) – peptone (0.2%) medium. Growth was evident within a few days of inoculation and eventually formed a mat completely covering the membrane. After several weeks' growth it had undergone the usual color changes observed when the fungus is grown in petri plates. At the end of 8 weeks the medium was changed to one containing only glucose (0.2%). The normal flow rate was maintained so that the nitrogen content of the medium in the cell dropped only slowly. When the culture was 9 weeks old, a rapid growth of synnemata began, the largest attaining a length of 20 to 25 mm within 10 days, after which lengthening ceased. However, new synnemata continued to appear and grow during the next few weeks. The experiment was concluded at the end of 12 weeks.

The basic design of this apparatus has proved satisfactory in use. The membrane is the critical component and only a proper choice will ensure successful results. An early idea was to use a semi-permeable membrane which would allow nutrients to diffuse through to the mycelium but would not allow the mycelium to enter the lower cell. Cast films of collodion (cellulose nitrate) and disks cut from dialysis tubing were both tried but were found unsatisfactory since the growth rate was considerably reduced. This fungus, although only weakly cellulolytic, did weaken the dialysis tubing material. This property also made the use of hardened filter paper unpractical. The present membrane is still not ideal because of its multilayer nature. If another fungus was to be cultured in this apparatus it would probably be necessary to use a different membrane than the one found best for *H. gigantea*.

One source of trouble was the tendency of the mycelium which reached the lower cell to be carried into the waste medium system where it could grow and block the flow of medium. This complication can be largely avoided by the proper choice of membrane but it is doubtful if it can be eliminated entirely. Hunt (2) and Elsworth *et al.* (1) have reported similar trouble in their growth apparatuses and the latter authors used a heating jacket on the medium line as a remedy.

The apparatus might also be improved by the use of a modified form of upper cell. The present design, because of the shoulder, causes some optical distortion, making direct observation of the culture difficult. A straight-sided upper cell, provided with a plate glass cover ground to fit and clamped in position, would greatly facilitate viewing. The inoculum and air supply could be introduced through a side arm or through a hole at one side of the glass cover.

The feed supply used at present is very simple in design and consequently limited in versatility, but it could be elaborated to any degree necessary. The addition of a motor-driven syringe for injecting special metabolites, as described by Perret (3), would facilitate certain types of studies.

Acknowledgment

This work was done under the direction of Dr. H. W. Buston in the Biochemistry Laboratories, Imperial College of Science and Technology, London, England.

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2. Hunt, P. Physiological aspects of the formation of sclerotia by *Rhizoctonia solani* and some other fungi. Ph.D. Thesis, Bristol University. 1957.
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SELECTIVE INHIBITION OF PHOTOSYNTHESIS[1]

MARCEL LEFRANÇOIS AND CYRIAS OUELLET

Abstract

In the presence of ethanol or acetone at concentrations up to 10% by volume, photosynthetic fixation of $C^{14}O_2$ by *Scenedesmus* proceeds at nearly normal or enhanced rates, over periods of several minutes, into glycolic acid, glycine, alanine, serine, and an unknown compound. However, fixation into other compounds, such as phosphoglyceric, phosphopyruvic, and aspartic acids and the lipids, is suppressed over induction periods of several minutes. The results indicate that blocking of the photosynthetic cycle unmasks an alternate path of fixation. It is suggested that the initial step is the carboxylation of phosphoenolpyruvic acid. At a concentration of about 10^{-5} *M*, *o*-phenanthroline enhances the fixation into compounds which are connected with the tricarboxylic acid cycle, at the expense of the fixation in all other compounds. With increasing inhibition, alanine is in all cases the last labelled compound to vanish, as if some of it had an origin independent of that of the other products.

Introduction

Experiments on the photosynthetic assimilation of $C^{14}O_2$ by *Scenedesmus* in the presence of methanol (11, 12) have shown that it is possible to preserve nearly normal rates of assimilation into several compounds, such as the simple amino acids, even when assimilation is practically suppressed in other compounds, including phosphoglyceric acid. A possible explanation of these results is that methanol inhibits the photosynthetic cycle (3, 7), thereby unmasking an alternate pathway which plays only a secondary role under normal conditions. It was suggested that the initial step in that pathway is the carboxylation of phosphoenolpyruvic acid.

The present paper gives an account of the effects observed under strong inhibition by ethanol, acetone, and *o*-phenanthroline.

Experimental

The methods used in these experiments were the same as in previous work (12) and the material was the same strain of *Scenedesmus*, obtained from General Biological Supply House, Chicago.

Briefly, each individual experiment was conducted as follows. From a freshly centrifuged, resuspended, and readapted culture of algae, a 30 cm^3 aliquot containing 0.2 mg wet algae was transferred to a flat bottle (10×7× 2.5 cm) and irradiated through a water screen from a photoflood RFL2 bulb at a distance of 15 cm. After 10 minutes' adaptation, the inhibitor was added 2 minutes before injection of 50λ of a solution of $Na_2C^{14}O_3$ containing 5 μc of C^{14}. The algae were killed suddenly by pouring into 110 cm^3 of boiling ethanol. Chromatography of the ethanol extracts and radioautography of the paper chromatograms were conducted as in earlier work (12) following essentially the indications given by Benson and his co-workers (5).

[1]Manuscript received February 19, 1959.
Contribution from the Département de Chimie, Université Laval, Québec, Qué.

Results

Inhibition by Alcohols and by Acetone

The total photosynthetic fixations of $C^{14}O_2$ in the presence of various alcohols, all at a concentration of 0.77 *M*, were compared in a series of experiments. Aliquots of the whole suspension of algae, killed in ethanol, including insolubles as well as solubles, were plated and counted. Figure 1 shows that the inhibiting power of these compounds increases regularly with the number of carbon atoms in the molecule. Although some of these alcohols may be utilized as food by the algae, the trend is that often found in the case of narcotics.

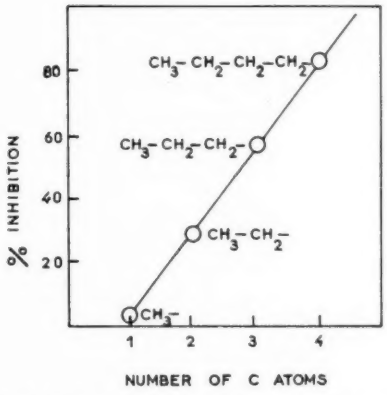

FIG. 1. Inhibition of over-all photosynthetic fixation of $C^{14}O_2$ by straight-chain alcohols, as a function of the number of carbon atoms.

In the following experiments with ethanol and acetone, the inhibitor was added to the algal suspension 2 minutes before injecting $Na_2C^{14}O_3$ and fixation was allowed to proceed over a period of 4 minutes. Fixation was totally suppressed only when the concentration of the inhibitor reached 12 to 15%. The products were analyzed by paper chromatography and radioautography.

Figure 2 shows the effects of various doses of ethanol (black circles) and acetone (crosses) on the labelling of several compounds. These effects are very similar to those of methanol (12). As the concentration of the inhibitor is increased, fixation into phosphoglyceric and phosphopyruvic acids decreases regularly; a similar trend is exhibited by malic acid, sucrose, and the lipids, and also by aspartic and fumaric acids (not shown in the figure). On the other hand, there is a marked stimulation of glycine and glycolic acid. Alanine, serine, and the unknown compound are strongly inhibited only at relatively high concentrations of the inhibitors. A noteworthy effect of acetone is the stimulation of succinic acid, an effect which is not observed in the presence of ethanol or methanol. The unknown compound is found on the chromatograms near the origin. It migrates a short distance (R_f about 0.1) in the phenol–water direction.

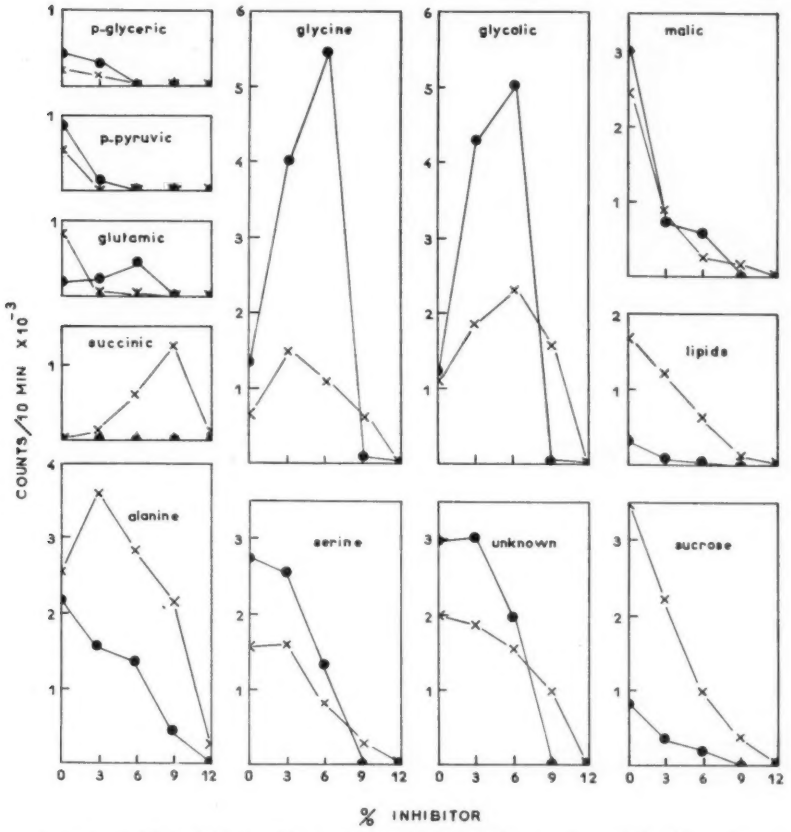

FIG. 2. Radioactivity fixed in 4 minutes PS into various compounds in the presence of ethanol (●) or acetone (×) at various concentrations (volume %). Radioactivities in counts × 10^{-3} per 10 minutes per 4 mg wet cells.

The kinetics of fixation was studied over periods of 0.5 to 8 minutes in three parallel series of experiments carried out with the same crop of algae within an interval of a few hours. It was thus possible to obtain a rough comparison of the rates in normal photosynthesis (control) and in the presence of 6% ethanol or 7% acetone. In all these experiments, the inhibitor was added 2 minutes before injecting $Na_2C^{14}O_3$. The kinetic curves (Figs. 3A and 3B) show the amounts of radioactivity incorporated into the compounds at various times in the absence of inhibitor (white circles) and in the presence of 6% ethanol (black circles) or 7% acetone (crosses). The effects are similar to those observed in the presence of methanol (12) for the following compounds: phosphoglyceric, phosphopyruvic, aspartic, and malic acids, sucrose, and the lipids. Ethanol accelerates early fixation into glycolic, glutamic, and succinic acids, and into glycine; acetone accelerates that into succinic acid only.

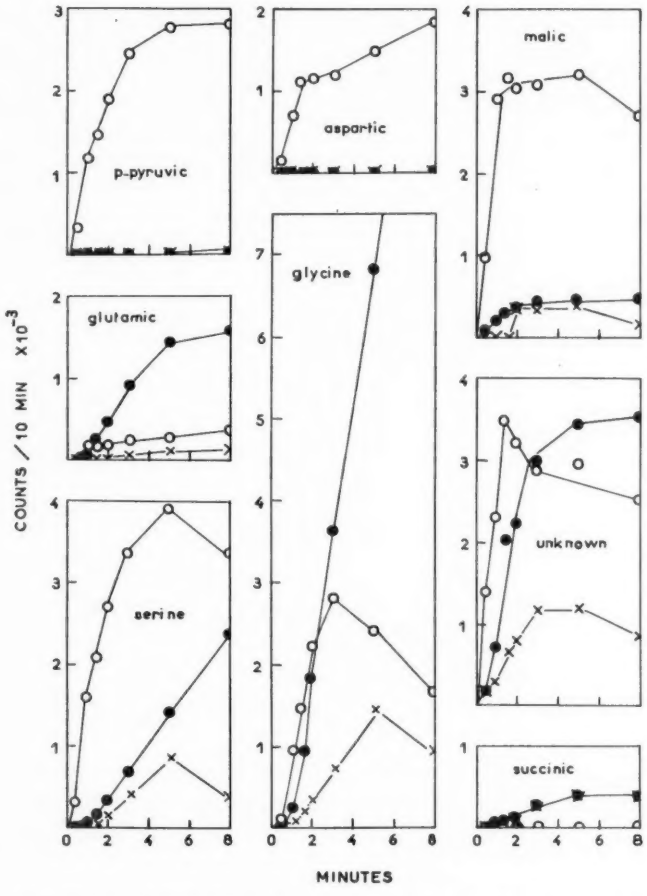

FIG. 3A. Kinetics of $C^{14}O_2$ fixation into various compounds in the absence of inhibitor (○) or in the presence of 6% ethanol (●) or 7% acetone (×). Radioactivities in counts × 10^{-3} per 10 minutes per 4 mg wet cells.

Figure 4 shows the relative (%) radioactivities at various times in the compounds which appear to be labelled early. In normal photosynthesis, these are phosphoglyceric and malic acids, and the unknown compound. In the inhibited reactions, the behavior of phosphoglyceric acid is quite different; fixation into this compound seems to be suppressed in 6% ethanol and shows an induction period of about one minute in 7% acetone. On the other hand, the curves for malic acid and for the unknown compound remain essentially unchanged; these compounds seem to preserve their priority in the presence of both inhibitors. The significance of these trends is restricted by the fact

that we have no data for periods shorter than 30 seconds. This consideration may affect the interpretation of an initially negative slope, but causes no ambiguity in the case of compounds which are not yet labelled after 30 seconds.

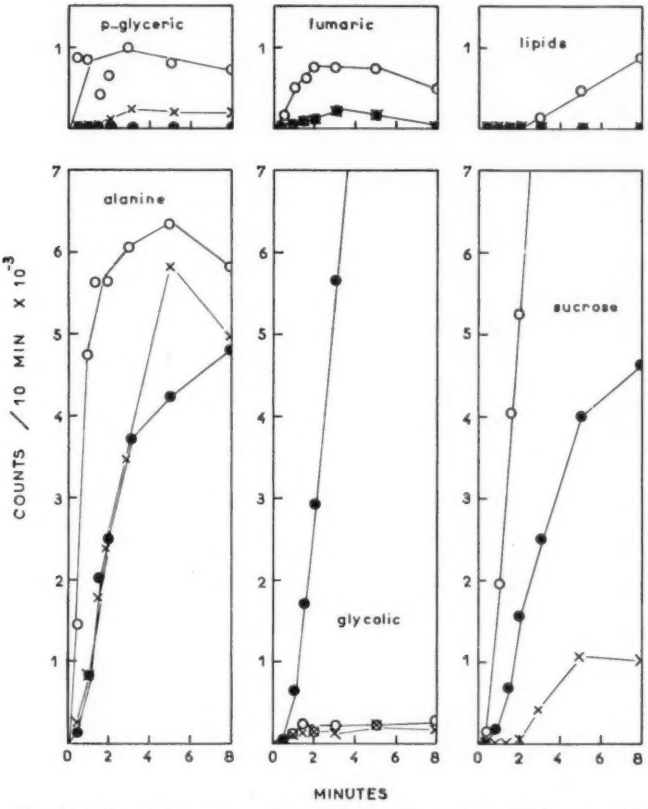

FIG. 3B. Kinetics of $C^{14}O_2$ fixation into various compounds in the absence of inhibitor (○) or in the presence of 6% ethanol (●) or 7% acetone (×). Radioactivities in counts × 10^{-3} per 10 minutes per 4 mg wet cells.

Inhibition by o-Phenanthroline

The chelating agent *o*-phenanthroline is known (13) to inhibit Hill's reaction. It was of interest to study its influence upon carbon dioxide fixation, where it should simulate a Mn^{++} deficiency. We found a mild stimulation of total fixation by this compound at 1×10^{-5} *M* and a 50% inhibition at about 2×10^{-5} *M*. Analysis of the distribution of the radioactivity fixed into the various compounds (Fig. 5) shows that the over-all stimulation observed at the lower concentrations is the resultant effect of strong stimulation of some intermediates and strong inhibition of some others. With increasing concentrations of *o*-phenanthroline, there is at first a considerable stimulation

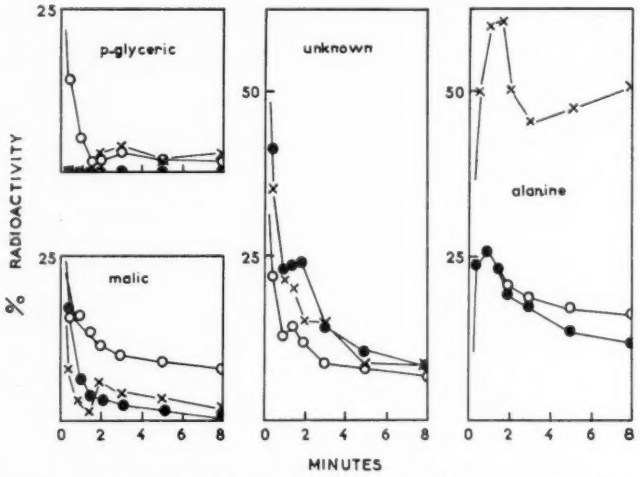

FIG. 4. Per cent of the total fixed radioactivity found in some compounds at various times in the absence of inhibitor (○) or in the presence of 6% ethanol (●) or 7% acetone (×). (From the data of FIGS. 3A and 3B.)

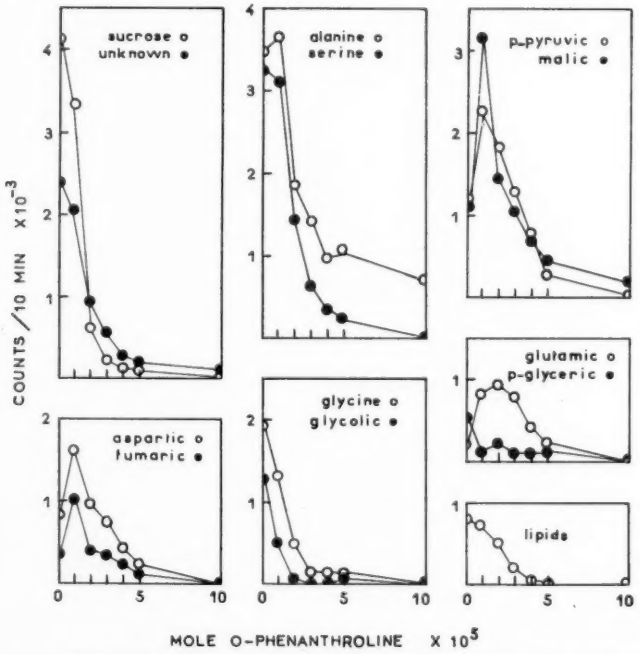

FIG. 5. Radioactivity fixed in 4 minutes PS into various compounds in the presence of *o*-phenanthroline at concentrations ranging from 0 to 10^{-4} mole per liter. Radioactivities in counts × 10^{-3} per 10 minutes per 4 mg wet cells.

of fixation in phosphopyruvic, malic, fumaric, aspartic, and glutamic acids. The curves for phosphoglyceric and glycolic acids and for glycine show a regularly increasing inhibition. Alanine, and serine, exhibit relatively flat curves which drop rapidly above a certain concentration of the inhibitor. These look like composite curves reflecting the sum of the two former effects. Note that phosphoglyceric acid, although strongly inhibited, is not suppressed by *o*-phenanthroline.

The Persistence of Alanine

It is noteworthy that whenever the dose of inhibitor is increased to the point where practically no photosynthetic fixation of radioactivity occurs, the residual radioactivity appears mainly and sometimes entirely in alanine. Table I shows the distribution of the radioactivity in experiments where inhibition was almost complete. The first column gives the radioactivities fixed in a typical control reaction lasting 4 minutes. These figures varied by about $\pm 10\%$ in experiments with different crops of algae. The following columns give the distributions of the radioactivities fixed photosynthetically in the presence of various inhibitors. In one case, inhibition was due to inactivation by ultraviolet irradiation of the algae during 40 minutes.

TABLE I

Persistence of alanine under conditions where C^{14} assimilation is almost completely inhibited. Radioactivities expressed in counts per 10 minutes per 4 mg wet cells

	Control	15% CH_3OH	15% C_2H_5OH	15% CH_3COCH_3	40 minutes U.V.	0.18 *M* CH_3CHO	10^{-4} *M* *o*-phenanthroline
Alanine	4,714	220	72	368	89	63	721
Malate	1,543	0	0	136	0	0	185
"Unknown"	2,965	0	0	0	0	116	89
Others	19,438	0	0	164*	0	0	0
Total	28,660	220	72	668	89	179	995

*Fumarate and succinate.

Behavior of the Amino Acids

If the radioactivity fixed in a given amino acid under given conditions is compared with the radioactivity fixed in the same acid in the control experiment, taken as unity, the numbers given in Table II are obtained. These values are larger or smaller than unity according to whether there is stimulation or inhibition. According to this "index", the amino acids are classified roughly according to the number of carbon atoms in the molecule. With *o*-phenanthroline, the relationship is the reverse of that which is observed with methanol, ethanol, or acetone as inhibitors. Although there are deviations, this trend was obeyed fairly well in most of our experiments. One explanation, among others, is that if a series of steps such as $C_2 \rightarrow C_3 \rightarrow C_4$ are

all similarly inhibited, C^{14} would tend to accumulate in the first compound and become rarer and rarer as it goes down the line. These compounds need not be the amino acids themselves.

TABLE II

Relative amounts of C^{14} fixed into the amino acids (compared with 1.00 for the control experiment) under various conditions

	Control	3% methanol	3% ethanol	3% acetone	10^{-5} *M* *o*-phenanthroline
Glycine	1.00	2.3	3.0	2.1	0.6
Serine	1.00	1.4	0.95	1.0	0.95
Alanine	1.00	0.75	0.8	1.35	0.8
Aspartic	1.00	1.1	0.25	0.0	1.7

Discussion

The observed patterns show that the substances used in these experiments inhibit water photolysis and the production of reducing power less strongly than they inhibit some later steps connected with $C^{14}O_2$ assimilation.

The alcohols and acetone behave like narcotics in their action on over-all $C^{14}O_2$ fixation; however, they also exert specific effects. Whatever the mode of action of ethanol and acetone, their effects, which are similar on the whole to those of methanol (12), exhibit two important features. First, it takes large concentrations of these substances, acting over periods of several minutes, to inhibit photosynthesis completely. Second, conditions can be obtained under which fixation of $C^{14}O_2$ into some key compounds, such as phosphoglyceric acid, is suppressed, whilst it proceeds at nearly normal or even enhanced rates into other compounds such as glycolic acid and the simple amino acids. The fact that, under such conditions, the rate of fixation in the dark remains negligible shows that the observed fixation is photosynthetic and not to be attributed to stimulation of metabolism by these compounds, which the cell presumably could use as food. The system behaves as if the main photosynthetic path were blocked and the plant made use of an alternate path, not readily distinguishable under normal conditions. Since malic acid retains its priority in time in the inhibited reactions (Fig. 4), it is suggested that the formation of this compound, presumably by carboxylation of pyruvic or phosphoenolpyruvic acid, becomes the main port of entry of $C^{14}O_2$.

Although malic acid is not part of the photosynthetic cycle (4), its early labelling has long been recognized (2, 6) as an indication that this or a closely related C_4 compound may be a primary product of photosynthesis. From our kinetic curves (Figs. 3A, 3B, and 4), it is seen that malic acid conserves its priority in the inhibited reactions, although most of the radioactivity accumulates after about one minute in other compounds such as glycolic

acid and glycine, in the presence of methanol and ethanol. It could be that the conditions favor a passage from malic to glyoxylic and acetic acids, following in reverse a path described by Kornberg and Krebs (9). This radioactivity would then flow into glycine and serine. This accumulation of radioactivity in the C_2 compounds is less pronounced in the presence of acetone than in that of the alcohols. Also, acetone allows some labelled phosphoglyceric acid to appear during the second minute, at which time there is also a rise in malic acid. In general, the effects of acetone seem to be somewhat less selective than those of the alcohols.

The chelating compound *o*-phenanthroline is known to inhibit photolysis, phosphorylation, and CO_2 fixation (1). However, it has been shown (10) that this inhibitor, at the concentrations used in our experiments, does not affect the carboxylation of ribulose-1,5-diphosphate in cell-free extracts. It can be seen on Fig. 5 that, at a concentration of 10^{-5} mole/liter, where it causes a mild over-all stimulation (about 10%), it brings about a large increase in the labelling of compounds which are related to the tricarboxylic cycle, at the expense of the other compounds. It should be interesting to investigate kinetically the action of this inhibitor in alternating periods of light and darkness, since it seems to interfere with the mechanism by means of which light is supposed to block the passage from the photosynthetic cycle to the respiratory cycle.

It is generally assumed that the radioactivity found in the amino acids in photosynthesis is that which flows from pyruvic acid through the tricarboxylic cycle. However, there are indications (12, 15) that this mechanism may not be sufficient to explain all the facts. Although our experiments were not designed to study this question, some of the results may have a bearing on it. From our kinetic curves, it appears unlikely that much of the serine labelled in the presence of 6% ethanol or 7% acetone comes from hydroxypyruvic acid; glycine is a more likely precursor (12). The early labelling of alanine and its remarkable persistence (Table I) suggest that this compound, while not a primary product (Fig. 4), is at least partly formed by a special mechanism. This is suggested by the singular resistance of alanine to *o*-phenanthroline (Fig. 5) and to the other inhibitors. For instance, one may assume that 10^{-4} *M* *o*-phenanthroline inhibits all the alanine which comes from pyruvic acid but is not sufficient to stop assimilation into the alanine which comes from the unknown source.

These and other observations (2, 3, 6, 8, 14, 15, 16) indicate that, although most of the $C^{14}O_2$ is assimilated through the photosynthetic cycle under normal conditions, other paths may be used at least over periods of several minutes.

Acknowledgments

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Résumé

En présence d'éthanol ou d'acétone à des concentrations allant jusqu'à 10% en volume, *Scenedesmus* peut fixer photosynthétiquement $C^{14}O_2$ à des taux normaux ou même accélérés dans l'acide glycolique, la glycine, l'alanine, la sérine et un composé inconnu, cependant que la fixation est supprimée durant plusieurs minutes dans d'autres composés tels que les acides phosphoglycérique, phosphopyruvique et aspartique et dans les lipides. Ces faits tendent à montrer qu'il y a blocage du cycle photosynthétique, ce qui démasque des voies alternatives de fixation. Comme étape initiale, on propose la carboxylation de l'acide phosphoénolpyruvique. À une concentration d'environ 10^{-5} *M*, l'*o*-phénanthroline stimule la fixation dans des composés liés au cycle des acides tricarboxyliques, aux dépens de la fixation dans tous les autres composés. Quand on augmente l'inhibition, l'alanine est toujours le dernier composé étiqueté à disparaître, comme si une partie de cette alanine avait une origine indépendante de celle des autres produits.

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A RARE SEED-BORNE DISEASE OF WHEAT CAUSED BY PODOSPORIELLA VERTICILLATA[1]

H. A. H. WALLACE[2]

Abstract

A rare fungus, *Podosporiella verticillata* O'Gara, was isolated from a single seed in each of 13 samples of common and durum wheat grown during the period 1946–1949 in western Saskatchewan and Alberta. The fungus had not been reported previously in Canada. *Helminthosporium cyclops* Drechsler is a synonym of *P. verticillata*. Naturally infected seed did not germinate. When mature seeds of cereals were artificially inoculated, germination was unimpaired and healthy plants were produced. Inoculation of developing kernels in heads of wheat and panicles of oats resulted in apparently healthy seeds, but when these were placed on moist filter paper they either failed to germinate or produced stunted seedlings. This injury is apparently caused by a toxin produced by the fungus. The fungus is not likely to be of economic importance under western Canadian conditions.

Introduction

In the autumn of 1946 an unfamiliar fungus (Fig. 1) was observed growing from an ungerminated kernel of wheat in a routine germination test conducted by the Line Elevators Farm Service, Winnipeg, Manitoba, Canada. This fungus, identified as *Podosporiella verticillata* O'Gara, was found in six samples of the 1946 crop and five of the 1947 crop, all originating in the area between Assiniboia, Saskatchewan, and Edmonton, Alberta. Since there was a possibility that the fungus might become an important seed-borne pathogen the author decided to study it in detail.

Morphological Studies

Appearance of the Fungus on Infected Seed

When naturally infected kernels of wheat are incubated on moist filter paper they fail to germinate, and if the incubation period is extended to 7 or 10 days, dark synnemata grow out from the kernel surface. These synnemata are simple or branched and may reach a length of 2.0 cm and a diameter of 1.0 mm. Their surface is black, with the exception of the growing tip, which is olivaceous. Internally the synnemata are white and pseudoparenchymatous. Growth is apical. It ceases under dry conditions but is resumed when moisture is supplied to the cultures. Usually there is no mycelium on the seed, although a mycelial weft does develop in some cases.

Conidiophores occur over most of the surface of the synnema except at the growing tip. Conidiophores are dark brown, 3–7 septate, and 70–130× 7–8 μ. Each conidiophore bears a cluster of conidia (average five) at its apex

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(Fig. 2). The conidia are cylindrical or tapering, 1–12 septate, 60–130×11–15 μ, and olivaceous to dark brown (Fig. 3). At the base of each conidium there is a conspicuous hilum.

Appearance of the Fungus on Artificial Media and the Effect of Various Factors on Growth

Mature conidia sown on wheat-meal agar germinate in about one hour. Germination is mostly bipolar (Fig. 3); intercalary germination is rare. On plain agar germination is mostly apical. The appearance of the colony varies with the medium. On potato-dextrose agar the fungus forms a dense, appressed, grey-green mat which gives rise to scattered, erect conidiophores, each bearing a short, spiral or verticillate cluster of conidia, very similar to those produced on synnemata. If this medium is acidified with lactic acid to a pH below 6.5, the colony develops a series of dark concentric zones, consisting of subspherical bodies resembling microsclerotia. Most of these bodies develop no further, but others grow apically to form synnemata similar to those formed on infected seed.

On plain agar and on Beijerinck's agar, made as described by Drechsler (3), growth is sparse, appressed or submerged, with the hyphae tending to be rhizomorphic, and the few scattered conidiophores develop conidia resembling those produced on potato-dextrose agar. In some cases the primary conidium on the conidiophores continues its apical growth, eventually producing lateral or terminal secondary conidia. After the colonies have grown for about 10 days, a few minute submerged black spherical bodies are formed in the agar. These bodies grow horizontally, very slowly; after about one month's growth they are 3 to 4 mm long. Some of them grow upward and emerge from the agar and form conidiophores near their apices. This would indicate that the submerged sclerotia, as they are called by Drechsler (3), are really undeveloped synnemata.

When the fungus is cultured on a medium containing 1.5% agar and 2.0% wheat flour, a transparent zone appears around the colony. When the medium is flooded with an aqueous solution of iodine in potassium iodide, the agar in the vicinity of the colony remains transparent, because of hydrolysis of the starch, while the remainder assumes a dark purple color.

Optimum lateral growth after 9 days on potato-dextrose agar occurred at 25° C. There was good growth at 30° C, but none at 35° C. The mycelium was not killed at 35° C, as cultures transferred from 35° to 25° C resumed normal growth.

Light does not appear to be essential for development of synnemata; synnemata formed readily on seeds buried in soil. Synnemata formed in culture are positively phototropic.

The occurrence of bacterial colonies on agar media adversely affected mycelial growth, but not the development of synnemata. The fact that infected seeds buried in soil produce synnemata, but no superficial mycelium, may be explained on this basis. The fungus invaded yeast colonies and produced numerous synnemata over them. There was a mutually antagonistic

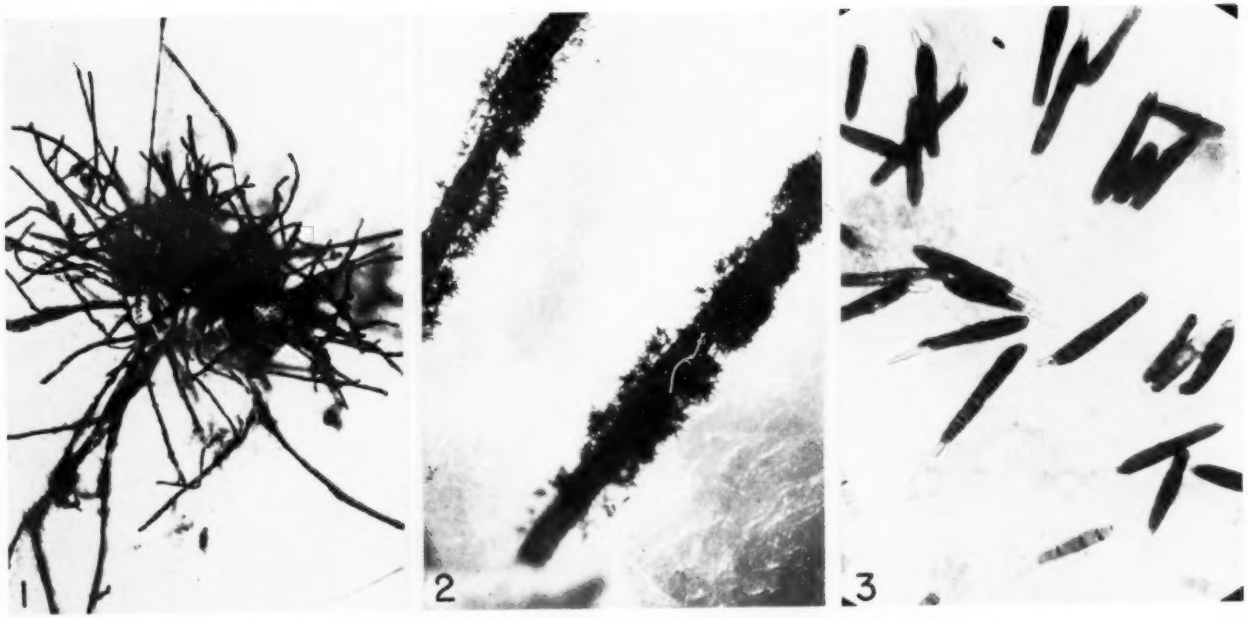

FIG. 1. A kernel of durum wheat infected by *Podosporiella verticillata* O'Gara. ×5.
FIG. 2. Sporulation on synnemata of *Podosporiella verticillata*.
FIG. 3. Conidia of *Podosporiella verticillata* germinating on potato-sucrose agar. ×150.

reaction between *P. verticillata* and *Fusarium culmorum*, and some unidentified species of *Penicillium*. *Trichoderma glaucum* grew readily over the surface of *P. verticillata* in culture, appearing to parasitize it.

Variation in Culture

Saltation in culture was frequent and varied. The saltants were relatively unstable, as reversals to parent type occurred. One saltant was sterile; no synnemata were formed and no conidia were borne on the mycelium.

Five naturally infected seeds, each from a different source, were placed on moist filter paper and produced some spores. An average of 10 single spore cultures was obtained from each infected seed. It was found that the cultures from each seed differed from the cultures isolated from each of the other seeds, but the cultures obtained from any one seed, with one exception, were very similar in type of growth, tendency to saltate, and zonation. The cultures of the fifth seed consisted of three distinct types, each of which differed from the types obtained from the other four seeds.

Taxonomy

The genus *Podosporiella* was erected by Ellis and Everhart (4), who placed it in the Phaeostilbeae–Phragmosporae. They described the genus, in part, as follows, "Stroma cellular, soft, not carbonaceous, short cylindrical." The type species, isolated from leaves of *Garrya veitchii* collected in California, was named *P. humilis*.

In 1915, O'Gara (6) described a new fungus growing on seeds of wheat attached to dwarfed plants growing in the Salt Lake Valley of Utah. He named the fungus *P. verticillata*. These two species are the sole members of the genus *Podosporiella*.

In 1919, Brittlebank and Adam (1) isolated a new fungus that caused a dwarfing of wheat and oat plants at Hopetown, Australia. They named the fungus, which belonged to the order Spaeriales–Phaeodictyae, *Pleosphaeria semeniperda*. The imperfect stage was said to agree closely with *P. verticillata*.

In 1920, Drechsler (3) isolated a new fungus from leaf lesions of *Danthonia spicata* (L.) Beauv., naming it *Helminthosporium cyclops*.

The writer (11), in 1950, drew attention to the resemblance of *H. cyclops* to the imperfect stage of *P. semeniperda* and to *P. verticillata*.

In 1952 Cooke and Shaw (2) reduced *P. verticillata* O'Gara and *H. cyclops* Drechsler to synonymy with *P. semeniperda* Brittlebank and Adam.

Neither Dorothy Shaw (7) working with Australian isolates of *P. verticillata*, nor the writer using Canadian isolates, has been able to induce the fungus to produce a perfect stage. Therefore, it is deemed advisable to retain *Podosporiella verticillata* O'Gara, which has priority of description, as the name for the imperfect stage of the fungus.

Pathogenicity Tests

O'Gara (6) stated that *P. verticillata* attacked the wheat kernel at or about the time of germination, completely destroying the contents, and as a

consequence the seedling was deprived of its normal food supply. He made no pathogenicity tests. Brittlebank and Adam (1) placed surface-sterilized wheat and oat seeds beside spore clusters of *P. semeniperda* in sterile soil. All seed germinated, but after 29 days the seedlings were stunted and the root bases were injured. Drechsler (3), who isolated *H. cyclops* from small lesions on dead leaves of *Danthonia spicata*, made no pathogenicity tests. The writer (11) isolated *P. verticillata* from wheat seeds that did not germinate.

P. verticillata has been reported on *Triticum* spp. from the United States (6), Australia (7), and Canada (11); *P. semeniperda* on *Avena* (1), *Bromus* (1), and *Triticum* (1, 5, 8); and *H. cyclops* on *Bromus* (2), *Danthonia* (3), *Poa* and *Triticum* (9), all from the United States, and *Trisetum* from Alaska (10). Pathogenicity tests were confined to cereals, but recently *H. cyclops* was isolated from seed of *Tragopogon dubius* Scop. (2) in the Chicoraceae indicating that it may have a wider host range than was previously suspected.

Infested Soil

Inoculum, prepared by growing *P. verticillata* on autoclaved wheat grains in flasks, was mixed with autoclaved soil. To provide a control, uninoculated, autoclaved wheat seed was also mixed with soil. Seeds of various cereals were sown in the soil and allowed to grow for 34 days in greenhouses kept at about 60° and 80° F respectively.

Seed germination and seedling vigor from infested and uninfested soil were similar. In one instance the hypocotyl of a barley plant was pierced by a synnema, but when the hypocotyl was surface-sterilized and plated no mycelium emerged from the tissues.

Seed Inoculation

Seeds of spring, winter, and durum wheat; oats; barley; winter rye; and corn were dipped in a spore suspension and planted in autoclaved soil. Six weeks later the seedlings were examined, and although the inoculated seeds bore typical synnemata, seed germination and vigor were equal to those of the controls.

The normal growth of the seedlings indicated that they were not starved as was suggested by O'Gara (6). The root bases were not injured as reported by Brittlebank and Adam (1) for *P. semeniperda*.

Leaf Inoculation

Spores of *P. verticillata* in water plus an equal volume of ground-up potato-dextrose agar were sprayed on the leaves of the same kinds of cereals as were included in the seed inoculation test. The sprayed plants were incubated in a moist chamber for 1 day and then transferred to a greenhouse bench at 60° F for several days. The fungus grew on droplets of the suspension on the leaves, but no lesions developed. A profusion of spores was produced on one dead leaf of oats.

Boot and Head Inoculation

Conidial suspensions were injected into the "boot" or sprayed on the inflorescences of wheat, oats, and barley. They were then covered with

cellophane cigar wrappers to prevent drying. The anthers were particularly susceptible, the infected tissues producing masses of conidia. The glumes, lemmae, and paleae of all cereals, and pedicels of oats, became infected as these parts matured. The seed appeared healthy, but inoculated wheat and oat seed incubated on moist filter paper either failed to germinate or germination was retarded. All seeds produced synnemata, and seeds with three or more synnemata always failed to germinate. Infected barley produced few synnemata on the seed, but the awns gave rise to conidia and, later, synnemata. Barley seed germinated normally.

Effect of Staled Culture Media on Seed Germination

The adverse effect of *P. verticillata* on seed germination could have been caused by a toxin. To test this possibility, the fungus was grown on a liquid medium composed of 2% wheat meal in 100 cc of distilled water. After 7 weeks' growth in 250-cc flasks, the cultures were filtered and a portion diluted to 2% with water. The diluted and undiluted filtrates respectively were dispensed in 8-cc aliquots over thin filter paper in Petri dishes, and 25 seeds of wheat were sown in each dish.

Germination counts were made after 5 days and the length of the shoots was recorded. Filtrates of each of several strains of the fungus retarded or inhibited germination to various degrees. There were significant differences in the shoot length between the strains, and all strains reduced the shoot length significantly below the check. The toxicity of the filtrates, which were alkaline (pH 7.5–8.0), was not affected by boiling.

When seeds sown in soil were "watered" with undiluted filtrate, prepared as above, there was no effect on germination or growth of the seedlings.

A preliminary test had indicated that a very dilute solution of the toxin might stimulate germination. Therefore, 3-week-old cultures, prepared by the same method, were used in another test. The dilute solutions were applied to seeds on filter paper as before. A germinating seed of wheat produces three seminal roots, then, a few days later, two more, and sometimes a sixth root is produced by the coleorhiza. The highly diluted filtrate stunted the first three seminal roots, but stimulated the earlier formation of the fourth, fifth, and sixth roots.

Control

Wheat seed, artificially infected with *P. verticillata* while it was still developing in the heads of wheat plants, and later harvested, was treated with an organic mercury seed dressing. Many seeds failed to germinate, and the non-germinating seeds produced synnemata. The effectiveness of other seed treatment chemicals was not tested.

Since inoculum is said to come from volunteer wheat plants (6) and grasses (1, 2, 3, 9), trash cover and "stubbled in" cereal crops probably increase the amount of the infection. Fall or spring plowing, which covers plant residues, should help to control the disease.

Discussion

The habit of growth of *P. verticillata* O'Gara is so unusual and its fruiting organs are so striking that it seems remarkable that it has not received more attention. This may be due to the fact that the diseases with which this fungus is associated have been of little economic importance. It is more likely, however, that it has escaped notice under field conditions because naturally infected seed often fails to germinate. The long incubation period before appearance of the fruiting structures on seed passed through routine germination tests may be the reason for its escaping notice by seed technologists.

Special environmental conditions are required to bring about infection of the seed. O'Gara (6) found the fungus where wheat was growing continuously and was self-seeding. Under such conditions inoculum would tend to increase and the chance of seed infection would be very good. In western Canada, where very short crop rotations are the rule, an accumulation of inoculum seems to be unlikely, unless the fungus invades the stubble which in many areas is left on the soil surface. It is possible that native grasses are the chief source of inoculum in this area.

The results obtained in the studies at the University of Minnesota and at Winnipeg suggest the following cycle of development for *P. verticillata*:

In autumn infected seeds, chaff, and straw drop to the soil surface and, if the weather is suitable, synnemata may be formed on them. The fungus can probably overwinter as synnemata or mycelium in seed or straw, since test tube cultures of the fungus were still viable after 7 months' exposure to severe winter temperatures in an open shed at Winnipeg. (They were still viable after 4 years at room temperature.) In the spring, the surviving mycelium likely produces a new crop of conidia and synnemata, while the surviving synnemata also resume growth and produce spores. During the early summer, dead leaves on seedlings and litter on the soil surface increase the inoculum. The spores so produced infect first the anthers, later the developing seed, and finally the chaffy parts of the head. Although the fungus seems to produce an exoenzyme dissolving the starch, and a toxin affecting the seed, neither of these substances affects seed development in infected heads.

Since the pathogen is not systemic, and since the synnemata formed on infected seeds do not seem to grow to a length of more than 2 cm, it is improbable that inoculum from infected seeds sown by a drill would reach the soil surface. The inoculum could be exposed in areas where soil drifting occurs. The principal source of inoculum would most likely be infected seed and straw from the previous crop scattered on the soil surface, or residues of grass hosts in the borders of the field.

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STUDIES ON SLIME ACCUMULATIONS IN PULP AND PAPER MILLS

III. THE STIMULATION OF GROWTH BY "WHITE WATER"[1]

D. BREWER

Abstract

In a basal medium which supported good growth of two species of fungi, incorporation of "white water" was found to increase their rate of growth. Preliminary efforts to separate the active agent have been carried out, and the presence of at least two substances has been demonstrated. One of them stimulated the growth of an isolate of *Phialophora fastigiata*, whilst the other stimulated the growth of an isolate of *Phoma* sp. The implications of the presence of these two substances in "white water" are considered.

Introduction

The recirculated process water from a pulp and paper mill was not capable of supporting growth of fungi isolated from pulp and paper mills, when tested under laboratory conditions. However, when this "white water" was incorporated into a basal medium which supported good growth of these fungi, the growth of many of the isolates was stimulated. This report is a record of work carried out to characterize the substance or substances which induce this stimulation.

Materials and Methods

The isolates used in these studies were as follows: 8-2E, *Phialophora fastigiata*, isolated from an accumulation of slime in the channel feeding the sulphite rotary screens in a mill in Quebec; 1-9E, *Phoma* sp. obtained from slime accumulating on the sulphite flat screens in a mill in New Brunswick.

The basal medium was as previously employed (1) with 2% glucose as the carbon source and ammonium tartrate as the nitrogen source.

The spore inoculum was obtained by flooding a 14-day-old culture of the organism on 2% malt agar with sterile distilled water and adjusting the spore concentration to 12×10^6 spores per milliliter. One milliliter of spore suspension was added to 50 ml of medium, and the flask incubated at 20° C in the dark on a rotary shaker for 4 days. The platform of the shaker had a displacement of 1½ inches and the revolutions were 240 per minute. The mycelium was obtained by filtering through a fine sintered glass filter and then dried at 105° C overnight. In all cases flasks were inoculated in triplicate. The different media after autoclaving had pH values between 5.1 and 5.5, except the basal medium plus white water treated with IR 120, which had a pH of 4.2.

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Experimental

Growth-Promoting Ability of "White Water"

The nutrients of the basal medium were dissolved in both distilled water, and in "white water" which had been filtered free of fibers. The amounts of growth of the isolates in the two media are shown in Table I. It is seen that in both instances the growth in the "white water" medium was very much greater than in the basal medium. The increase in growth was almost 25-fold in the case of isolate 1-9E.

TABLE I

The growth of the two isolates in the basal medium, the basal medium plus "white water", and basal medium in "white water" treated in various ways, expressed in milligrams of mycelium (dry weight)

	8-2E	1-9E
Basal medium	44	12
Basal medium plus "white water"	145	299
Basal medium plus acid hydrolyzate of "white water"	77	190
Basal medium plus alkali hydrolyzate of "white water"	94	191
Basal medium plus chloroform extract of "white water" residue	138	trace
Basal medium plus ether extract of "white water" residue	191	trace
Basal medium plus acetone extract of "white water" residue	367	13
Basal medium plus ethyl alcohol extract of "white water" residue	432	115

Stability of the Growth-Promotion Ability of "White Water" to 0.1 N H_2SO_4 and 0.1 N KOH at 121° C for 20 Minutes

Three hundred milliliters of "white water" were evaporated to dryness *in vacuo* at 60° C, and the residue dissolved in 25 ml of 0.1 *N* H_2SO_4. This was autoclaved at 121° C for 20 minutes, then adjusted to pH 7. The volume was increased to 300 ml with distilled water and the basal nutrients added. Another 300-ml aliquot of "white water" was similarly treated using 0.1 *N* KOH. Flasks of these media were inoculated in triplicate with the two isolates. Table I shows the amount of growth in these media after 4 days' incubation. It was found that in both instances the growth-promoting ability was partially destroyed by the treatment.

Extraction of the Growth-Promoting Ability from "White Water" Residue by Organic Solvents

Three hundred milliliters of "white water" were evaporated as before and the residue shaken for ½ hour with 100 ml of ether. The ether solution was filtered and then evaporated to dryness and the residue dissolved in 300 ml of distilled water. Nutrients of the basal medium were added to this solution and the resulting medium assayed for ability to promote growth in the normal way.

Other 300-ml aliquots of "white water" were treated in a similar way, with either chloroform, acetone, or anhydrous ethyl alcohol.

The results as compared with the basal medium and the basal medium in "white water" are shown in Table I.

In the case of isolate 8-2E, it was found that both chloroform and ether could be used to extract the growth-promoting substance from the "white water" residue. The amount of stimulation was approximately the same as with the original "white water". However, the amount of growth in the media containing the acetone and alcohol extracts is more than twice that in the medium with the "white water".

Isolate 1-9E behaved differently from isolate 8-2E in these media. No stimulation was observed in the medium with the acetone extracts and a suppression of growth occurred in the media with either the chloroform or the ether extracts of "white water" residue. The stimulatory substance appeared to be slightly soluble in ethanol.

Absorption of Stimulant in "White Water" by Ion Exchange Resins

Three hundred milliliter aliquots of "white water" were shaken with an excess of either IR 120 or IR 45 ion exchange resins, filtered, and the nutrients of the basal medium were added. The resulting media were assayed for the presence of the growth-promoting ability in the usual way. The results are tabulated in Table II. It was found that the cationic exchange resin removed part of the stimulatory ability of "white water" for both organisms. The amount of growth of isolate 8-2E in the medium containing the "white water" treated with the anion exchange resin was greater than in the medium containing the "white water". The anion exchange resin appeared to remove most of the stimulatory power of "white water", when considering the isolate 1-9E.

TABLE II

Effect of treatment with ion exchange resins on the stimulatory power of "white water" expressed in milligrams of mycelium (dry weight)

	8-2E	1-9E
Basal medium	57	15
Basal medium plus "white water"	208	270
Basal medium plus "white water" treated with IR 45 (anionic)	348	192
Basal medium plus "white water" treated with IR 120 (cationic)	134	97

Absorption of Stimulant by Activated Charcoal

One thousand milliliters of "white water" were concentrated *in vacuo* to 20 ml and then shaken with well-washed activated charcoal. The liquid was then filtered free of charcoal and 1 ml added to each of six flasks, to which had been added 50 ml of basal medium. By this means the components of "white water" not absorbed by charcoal are present in the basal medium to approximately the same concentration they were in the original "white water". The resulting medium was assayed for stimulatory power and compared with that of the original "white water". The results are shown in Table III. It was found that the stimulant in the "white water" was removed completely by activated charcoal for both 8-2E and 1-9E.

TABLE III

Effect of treatment with activated charcoal on the stimulatory power of "white water", expressed as milligrams of mycelium (dry weight)

	8-2E	1-9E
Basal medium	65	10
Basal medium plus "white water"	146	174
Basal medium plus "white water" treated with activated charcoal	62	6

Discussion

The appreciable diminution in the stimulatory power of the "white water" after treatment with either acid or alkali suggests a partial hydrolysis of one or more substances in the "white water".

The presence of at least two substances in "white water" which can stimulate the growth of certain fungi was shown by the series of experiments with organic solvents. The stimulant for isolate 8-2E is soluble in alcohol, acetone, ether, and chloroform, whereas that stimulating 1-9E is not soluble in acetone, ether, or chloroform. The increase in stimulation of isolate 8-2E by the acetone and ethanolic extracts over that of the "white water" can be explained by the presence in the "white water" of an inhibitor, which is not soluble in acetone and alcohol. This inhibitor may be soluble in chloroform and ether and thus the stimulating power of these extracts would be approximately the same as the original "white water". That there was an inhibitor, soluble in chloroform and ether and not in acetone and ethyl alcohol, is suggested by the decrease in growth of isolate 1-9E in media containing these extracts as compared with the basal medium. The lower stimulation of the isolate 8-2E observed in the chloroform and ether extracts as compared with the alcohol and acetone extracts could be explained also by either that the stimulatory substance was less soluble in chloroform and ether and thus there was incomplete extraction or that there are two substances capable of exerting a stimulatory effect, one of which is soluble in ether and in chloroform.

By the stimulation of growth of isolate 8-2E, it was seen that some of the stimulatory power of "white water" was removed by the cation exchange resin, but that an inhibitor was removed by the anion exchange resin with the result of greater stimulation. The cation exchange resin removed a large amount of the stimulatory power of the "white water" for isolate 1-9E, but the anion exchange resin also removed a certain amount. This suggests that there may be two substances capable of exerting a stimulatory effect on the isolate 1-9E.

The existence of more than one stimulant in "white water" and the difference in their effects upon two different organisms suggests that they may play an important role in the selection of the fungi that grow in different mills. It also suggests an explanation for radical changes in the fungal floras at a specific location of slime accumulation, when there has been no apparent change in conditions of growth. That the stimulatory substances originate

from wood is very probable. Robbins and Hervey found that the growth of some Basidiomycetes was stimulated when a liquid basal medium was supplemented with an extract from wood (2). It is possible that the amounts of each present in the "white water" could vary from time to time and that the proportion of each present at a certain time might determine, to a large extent, the predominant species in a slime accumulation.

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INFLUENCE OF GIBBERELLIC ACID ON THE BREAKDOWN OF NICOTINE IN TOBACCO[1]

E. V. PARUPS

Abstract

Detached leaves, leaf disks, and sterile, detached roots of tobacco were sprayed, vacuum-infiltrated, incubated, or treated with gibberellic acid to test the influence of this chemical on the breakdown of nicotine. No statistically significant differences in nicotine content were observed due to these treatments.

Introduction

It was recognized as early as 1943 by Yabuta (7) that foliar applications of gibberellic acid (GA) decreased the nicotine content of tobacco plants. This was confirmed by Parups (3) and Burk and Tso (1). Parups (3) suggested that GA caused changes in the metabolism of the tobacco root thus decreasing the nicotine content. It was also suggested that the possibility of nicotine breakdown in the presence of GA should be investigated. This paper is a report of an investigation of this problem.

Materials and Methods

Two experiments conducted on field-grown cigar tobacco var. Resistant Havana 211, were replicated six times, each treatment consisting of five or six detached leaves. The leaves were collected randomly from the upper half of the plants and brought into the laboratory. In experiment 1 both sides of the leaves were sprayed until dripping with an aqueous solution of GA at three concentrations: 0.0, 5.78×10^{-4}, and $1.15\times10^{-3}M$. They were then kept with the basal portion in distilled water for 1 week at room conditions.

In experiment 2 the leaves, collected as previously, were kept for 1 week at room conditions with the basal portion in an aqueous solution of GA at a concentration of $2.89\times10^{-4}M$. Controls were kept in distilled water. For both experiments a representative sample was dried immediately after the leaves were collected to serve as a reference for the nicotine content at start. At the termination of the experiments leaves were dried in a forced draft oven at 70° C, ground in a Wiley mill, and analyzed for nicotine by the method of Cundiff and Markunas (2).

In experiments 3, 4, and 5 leaf disks of greenhouse-grown cigar tobacco, var. Resistant Havana, cut with a No. 12 cork borer, were used. In all these leaf disk experiments the disks were taken at random and replicated 10 times. At the start of each experiment a representative number of disks was dried and weighed for estimation of the dry weight of the disks used, as well as for

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Contribution from the Tobacco Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ontario.

calculation of the initial percentage of nicotine. In experiment 3, 20 disks per treatment were placed in test tubes and vacuum-infiltrated with 25 ml of an aqueous solution of GA at five concentrations: 0, 10^{-6}, 10^{-5}, 10^{-4}, and $10^{-3}M$. In experiment 4 the possible breakdown of added nicotine was tested: 20 disks per tube were vacuum-infiltrated with 25 ml of distilled water only, or 25 ml of aqueous solutions of GA and nicotine of the following concentrations: (*a*) GA, 10^{-5} *M*; (*b*) GA, 10^{-5} *M* plus nicotine, 10^{-4} *M*; and (*c*) nicotine, 10^{-4} *M*. In both experiments the tubes containing liquid and disks were kept at room temperature for 20 to 24 hours.

In experiment 5 leaf disks were incubated in GA made up in one-half strength Vickery's solution as used by Stephenson (5). Incubation was carried out in Petri dishes containing a disk of 9 cm washed filter paper to spread the solution evenly. Each dish contained 20 disks upper side up and 10 ml of solution of 0, 10^{-6}, 10^{-5}, or 10^{-4} *M* of GA. The covered disks were placed below fluorescent light to give approximately 600 ft-c at the disk level. The leaf disks, paper, and solution of each dish were analyzed for nicotine after 1 week of incubation.

In experiment 6 the detached roots of *Nicotiana rustica* L. were grown in modified White's medium by the method of Macdowall.[2] Twenty-four flasks of 1- or 2-month-old root cultures were divided in two equal groups, depending upon their growth, by visual comparison. One group of 12 flasks was treated to give a final molar concentration of 10^{-5} of GA in the growth media. The other group was maintained as a control. The duration of this experiment was 2 weeks. In all leaf disk and root experiments the nicotine was determined by methods of Willits *et al.* (6).

Results and Discussion

The treatments and results of these experiments are presented in Table I. In experiment 1 the GA treatments decreased the nicotine content slightly but non-significantly. In experiment 2 the differences in nicotine content between the treated and untreated leaves were almost non-existent. In experiment 3 only the highest concentration of GA of 10^{-3} *M*, or 346 p.p.m., produced a slight and non-significant decrease in nicotine content. Additions of GA did not influence the content of added nicotine, as shown in experiment 4. In experiment 5 the incubation of leaf disks in light with nutrient solution and GA did not decrease but even slightly increased the nicotine content. Again the differences were statistically non-significant. In experiment 6 no decrease of the alkaloid occurred in sterile, detached tobacco roots when GA was supplied after the roots were developed and nicotine was formed. The growth of such roots is usually stimulated and the nicotine formation hindered by additions of GA (3). The average dry weights of these root cultures were: 0.02605 g for the checks and 0.02354 g for the treated ones. The growth of isolated tobacco roots has been shown to be directly related with nicotine production (4), thus explaining the slightly higher nicotine content of the check.

[2]F. D. H. Macdowall, unpublished data.

TABLE I

Nicotine content of tobacco leaves, leaf disks, and roots treated with gibberellic acid or nicotine

Experiment	Initial content of nicotine, %	Treatments, *M*	Nicotine, %	L.S.D. 5%
1	2.189	GA, 0	2.512	N.S.
		GA, 5.78×10^{-4}	2.472	
		GA, 1.15×10^{-3}	2.275	
2	2.069	GA, 0	2.557	N.S.
		GA, 2.89×10^{-4}	2.536	
3	2.836	GA, 0	3.093	N.S.
		GA, 10^{-6}	3.390	
		GA, 10^{-5}	3.177	
		GA, 10^{-4}	3.291	
		GA, 10^{-3}	2.848	
4	1.246	GA, 0	1.860	N.S.
		GA, 10^{-5}	1.755	
		GA, 10^{-5} + nicotine, 10^{-4}	5.536	
		Nicotine, 10^{-4}	5.810	
5	3.312	GA, 0	3.966	N.S.
		GA, 10^{-6}	4.038	
		GA, 10^{-5}	4.460	
		GA, 10^{-4}	4.296	
6	—	GA, 0	1.683	N.S.
		GA, 10^{-5}	1.558	

Examination of the numbers in Table I indicates that once nicotine has been formed applications of GA do not increase or decrease its content significantly. This supports the view that the characteristic decrease in nicotine content of tobacco, related to GA treatments, is due to changes in the producing metabolic system and is not caused by increased breakdown of the alkaloid.

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CYTOGENETIC STUDIES IN THE GENUS HORDEUM

I. CHROMOSOME MORPHOLOGY[1]

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Abstract

The morphology of the chromosomes of 16 *Hordeum* species was studied in considerable detail. No obvious trend in evolution is apparent from a study of the karyotypes of the species nor are any definite relationships with common barley (*H. vulgare*) indicated, except for two species, usually considered as being related, *H. spontaneum* and *H. agriocrithon*. On the basis of similarities of karyotype and morphological features of the plants seven groups were formed in which relationships are evident. The groups are: vulgare, marinum, murinum, californicum, stenostachys-pusillum, bulbosum, and jubatum. The plants within each group are identified by assigned numbers and by the specific names under which they were received. Within these groups are chromosome numbers of 14 and 28 and in one case 28 and 42. Allopolyploidy is indicated for most groups but *H. bulbosum* exists both as a diploid and as an autotetraploid.

Introduction

Since 1952 research in the genus *Hordeum* has been carried out by the barley and cytogenetics units of the Cereal Crops Division at Ottawa. A collection of many *Hordeum* species is being maintained and the studies include relationship with common barley, *H. vulgare*, cross compatibilities, polyploidy, and interspecific hybridization of the species. The morphology of the chromosomes of some of the species will be described in this paper.

Previous reports on the morphology of the chromosomes of the *Hordeum* species have mostly been confined to common barley and here the sizes and shapes of the chromosomes are fairly well known (4, 7, 12). Several other reports have dealt with one or two of the species (1, 10) but at the present time there is still only a limited amount of information available about chromosomes in the genus. There is even confusion regarding the chromosome number of some species. This stems from incomplete surveys, polyploidy in species accompanied by only minor changes in morphological characters, and difficulties in classification.

Some of the plant introductions reported on in this paper can be identified with reasonable certainty; others resemble known species and conform to established taxonomic descriptions but have a different chromosome number than is usually ascribed to that species. The short description given to identify the plants is not intended as a taxonomic description. Further, in the discussion no argument is raised to support or deny the existence of chromosome races within a species. The major concern of this paper is to show the similarities or dissimilarities of the chromosome morphology (karyotype) of several species and suggest relationships from this analysis.

[1]Manuscript received February 9, 1959.
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Methods

All studies of the morphology of the chromosomes have been made from metaphase of mitosis in root tips. Before fixation the roots were treated with a drug to shorten the chromosomes and destroy the spindle. The schedule is as follows:

1. Pretreat root tips for 3 hours in an aqueous solution of monobromonaphthalene.
2. Fix in 2BD; add equal parts of 1% chromic acid and let stand overnight.
3. Wash; then clear and soften roots in a mixture of equal parts of 3% hydrogen peroxide and a saturated solution of ammonium oxalate for 20 minutes.
4. Hydrolyze in 1 *N* hydrochloric acid for 20 minutes and stain in leucobasic fuchsin (Feulgen).
5. Squash in acetocarmine.

After this treatment, centromeres and nucleolar constrictions in the chromosomes are visible. Other secondary constrictions are usually not apparent (yet see section under *murinum*). Not all cells in a root tip are affected to the same degree and in some the chromosomes are more shortened than others (compare Figs. 1 and 2). If overcontracted the nucleolar arms are not always obvious. To be sure of the morphology of the chromosomes, cells at various stages were studied; the drawings were made of the most suitable and, as nearly as possible, the same stage for all species.

In the description of the chromosomes, those having a constriction subtending a nucleolus organizing region, i.e., those having a satellite, are called sat.-chromosomes; those with the centromere in a median, submedian, or subterminal position are called median, submedian, etc., rather than the longer but more correct designation each time.

Material

There is a great deal of confusion in the classification of the *Hordeum* species. Many synonyms are in use, several invalid names are commonly applied, and some species have been described and named that differ from other species by only minor characters. Then too, some species have been described and given new names when there are older valid names in existence. The Botany Unit, Botany and Plant Pathology Division, Science Service, Ottawa, has recently given this taxonomic problem specific attention and it is hoped that shortly information will be published clarifying the species. It is understandable that until all data are collected no definite commitments can be made by that unit. Therefore, for this and subsequent reports, rather than perpetuate an incorrect or invalid name for some of the material, all plants will be designated with the Cereal Crops Division "B" number, the source of origin will be noted, and the probable species name given. No authority for the name will be cited unless the sample was received with one. Specimens of all plants described are retained in our collection under our own number. Herbarium samples for most have been made by the Botany Unit.

The main characteristics of *Hordeum* are that the spikelets have one flower (floret) and there are three spikelets at each rachis node. Each floret is enclosed within two glumes. The species within this genus are often distinguished by the differential development of the glumes, awns, and other floral parts. In most species only the central floret is fertile, the two laterals are sterile. It has also been customary to describe plants as annual or perennial. Such a classification has not been applied here because under our conditions all species can be propagated by cloning and many species referred to as annuals have been grown for 3 or 4 years.

Results

Vulgare (sativum) Group

As used here the name *H. vulgare* includes the cultivated barley varieties in commercial use whether 2-rowed or 6-rowed. Further, no distinction is made for hooded or naked barley or other forms with head irregularities that are perhaps botanical curiosities but not deserving of a specific name. No Cereal Division "B" numbers are listed for the barley varieties studied but seed will be sent on request.

These studies confirm those of other workers previously mentioned. There are two pairs of sat.-chromosomes and the other 10 chromosomes are either median or submedian with not much difference in length. There are no definite subterminal chromosomes (Fig. 1). Contrary to some recent reports no extra constrictions were found in the chromosomes. In particular it is difficult to reconcile my results with those of Sharma (11), who found many constrictions in the chromosomes and suggested they may be all nucleolar organizers.

Two other species, *spontaneum* and *agriocrithon*, were found to have the same karyotype as *H. vulgare*. Samples of the former are: B164, received as *H. spontaneum nigrum* from Max-Planck Institut, Berlin; and B397, received as *H. spontaneum* C. Koch from the University Botanical Garden, Uppsala, Sweden. This species is very much like 2-rowed barley but has a distinctly brittle rachis. It has often been described as one of the progenitors of normal barley. B397 has yellow seeds and B164, black. The entries of *agriocrithon* studied are: B209, received as *H. agriocrithon* Åberg from the Botanical Gardens, Berlin–Dahlem; and B210, received as *H. agriocrithon* Åberg from Kew Gardens, and reported as being collected in Tibet. This species is similar to *spontaneum* except that the lateral florets are fertile. The seeds are black. The characteristic of seed production in the lateral florets is somewhat unstable, however, because on one occasion a plant produced both 2-rowed and 6-rowed spikes and even heads that were half 6-rowed and half 2-rowed.

Because of the similarity in morphology of the plants it is not surprising that the chromosome karyotype is the same for all three species. The existence of differing karyotypes in some varieties of barley cannot be excluded, but from my experience it is rather doubtful except in some translocation lines as shown by Hagberg (3).

Marinum Group

The plants of this group, known as Sea barley, are short, strongly branched with geniculate stems; spikes are usually not more than 6 cm with a tough rachis; the pedicels of the lateral florets are thickened and spreading; glumes of the central floret are rough, thick, spreading, and awl-shaped. The glumes of the laterals are also stout but in some plants the inner glumes of the lateral florets are very much flattened and thus lanceolate. This characteristic is used by many authors to distinguish two species in the group (5, 6). Covas (2) also distinguished two species on this basis but warned that intermediate forms are sometimes found. He also stressed that, "only cytogenetic evidence can decide whether the two entities are or are not conspecific." The specific names in this group are: *marinum*, *maritimum*, *hystrix*, and *gussoneanum*. Chromosome numbers of $2n = 14$ and $2n = 28$ have been reported.

Diploid species studied are: B51, received as *H. marinum* from Argentina; B205, and B908, received as *H. maritimum* var. *gussoneanum* from Botanical Gardens, Coimbra, Portugal; B254, received as *H. hystrix* from the Herbarium, University of California, Davis, California; B389, received as *H. marinum* Huds. from University Botanical Garden, Uppsala, Sweden; B863 and B963, received as *H. maritimum* ssp. *eumaritimum* from Botanical Institute, Strasbourg, France; B833, received as *H. hystrix* from Botanical Gardens, Kew; B1039, received as *H. maritimum* from Liége, Belgium, through the Botany Unit of Science Service, under No. 2058.

The morphology of the chromosomes of the diploid group is shown in Figs. 3 and 7. The most conspicuous chromosomes are: one pair of sat.-chromosomes; one pair of subterminal chromosomes; and one pair of median and two pairs of submedian long chromosomes. The other chromosomes are shorter, either median or submedian. Although metaphases in the roots of over 40 plants from the diploid marinum group were studied in detail, no differences between plants could be detected in sizes and shapes of the chromosomes. Data on the morphology of the plants and growth habit features are not available for all entries but there are some definite differences in some of the characteristics that have been used to delineate species, e.g., in B51 and B389 the inner glume of the lateral floret is strongly widened but in B205 and B833 it is not; there are differences in maturity between the species B51, B205, and B254. Some samples have spikelet groups with seed set in the lateral florets. It is difficult to separate species on these features. On the basis of morphology of the chromosomes it is not possible to separate them.

Tetraploid species are: B665, received as *H. gussoneanum* from Turkey through the Botany Unit, under No. 2004; B1038, received as *H. marinum* from Australia through the Botany Unit, under No. 1969.

The morphology of the chromosomes of the tetraploid group is shown in Figs. 4 and 8. It is clear that the plants are not autotetraploids—only one pair of sat.-chromosomes is visible. It is also possible to pick out some chromosomes that resemble those of the diploid although others are not

FIGS. 1–6. Photomicrographs of root tip metaphases, 1200×.
FIG. 1. *H. vulgare* ($2n=14$), 2 pairs of sat.-chromosomes.
FIG. 2. *H. agriocrithon* ($2n=14$) (B210), an identical karyotype to *H. vulgare* but at an earlier phase of division and less contracted.
FIG. 3. Marinum group ($2n=14$) (B389), one pair of sat.-chromosomes.
FIG. 4. Marinum group ($2n=28$) (B665), one pair of sat.-chromosomes.
FIG. 5. *H. murinum* ($2n=28$) (B253), three pairs of sat.-chromosomes, note one in center of field.
FIG. 6. *H. californicum* ($2n=14$) (B257), sat.-chromosomes at seven and six o'clock, note also chromosomes with a subterminally attached centromere.

identifiable. One pair of short subterminal chromosomes is easily distinguished from all the chromosomes of the diploid set.

In view of the existing difficulties in classification it is not surprising that the karyotype is similar for the various diploid species. The question posed by Covas (2) can therefore be answered. The morphology of the chromosomes indicates that all the diploid species of the marinum group are conspecific. The tetraploid species present another problem similar to that outlined in the murinum group (see murinum section) where plants have two different chromosome numbers and two distinct karyotypes but are so similar in appearance that they usually cannot be distinguished except by the study of the chromosomes. If plants are allopolyploids the other parental form may be isolated after a thorough crossing program.

Murinum Group

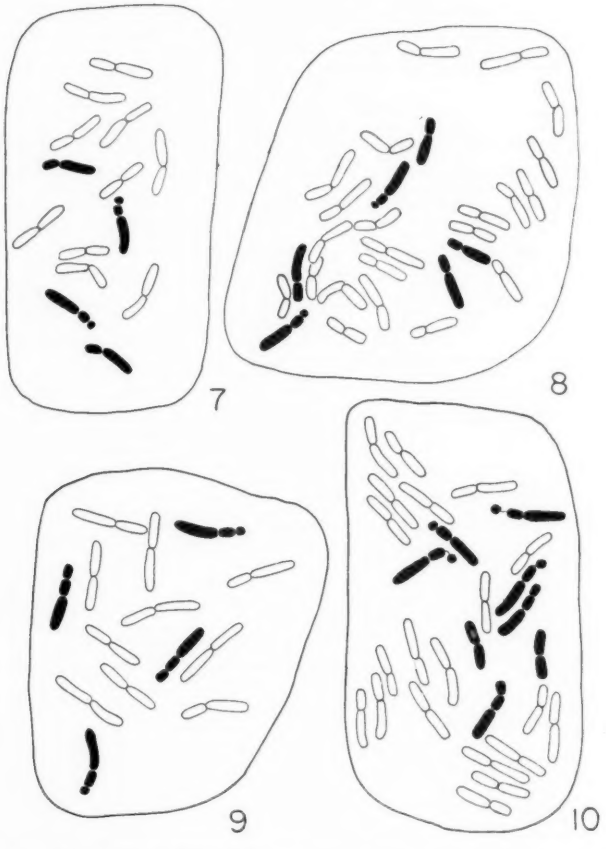
In this group are vigorous plants which characteristically inhabit waste ground. This weed varies from a few centimeters to a meter high depending upon conditions of growth. The spikes are flattened and may be 3–10 cm long; the lateral florets are sterile and spikelet groups fall at maturity. The distinguishing feature of this group is that the glumes of the central floret and inner glumes of the laterals are awl-shaped and conspicuously ciliate. In a recent paper Morrison (9) pointed out some of the difficulties in classification of the species *H. murinum*, *leporinum*, *stebbinsii*, and *glaucum*. The chromosome numbers are $2n = 14$ and $2n = 28$.

Diploid specimens examined are: B330, received as *H. leporinum* Link from Botanical Gardens, Moscow; B499, B500, B502, received as *H. stebbinsii* from Botany Unit, under No. 1900, 1901, and 1903 respectively; B600, received as *H. glaucum* Steud. (*H. stebbinsii* Covas) from University of California, Davis (spikelets are black); B1040, received as Wild Hordeum from U.S.D.A. and collected in Arizona.

The chromosomes of the diploid group are quite similar to those of common barley in so far as most chromosomes are median or submedian with not much difference between the shortest and the longest. There are two pairs of sat.-chromosomes (Fig. 9) that show up clearly, one pair with a much larger satellite than the other. The other chromosomes are either submedian or near-median.

Tetraploid specimens examined are: B52, received as *H. murinum* from Argentina; B241, received as *H. murinum* from the Botanical Institute, Udine, Italy; B253, received as *H. stebbinsii* from the Herbarium, Davis; B366, received as *H. murinum* from University Botanical Gardens, Coimbra, Portugal; B381, received as *H. murinum* from Odessa and B382, received as *H. murinum* from Glasgow, before 1951; B514, received as *H. murinum* from Liége; B649, received as *H. murinum* L. from Botanical Gardens, Angers, France; and B1041, received as *H. murinum* from the Botanical Institute, Vacratot, Hungary. In addition there are specimens from a former study preserved at the Herbarium, Leiden, and with the Botany Unit at Ottawa.

The chromosomes of the tetraploid group have been described previously (9). At that time the diploid complement had not been studied in detail. Now a more critical study has been made in many root tips of the tetraploids and a correlation must be made in the karyotype. There are three pairs of sat.-chromosomes in the plants with 28 chromosomes. The six chromosomes with satellites are not always visible in every cell but in a good preparation are quite clear (Figs. 5 and 10). Two pairs have the small satellite and in one pair the constriction is almost midway between the centromere and the end.



FIGS. 7–10. Drawings of root tip metaphases, chromosomes spaced out in drawing, sat.-chromosomes and other distinctive chromosomes in solid black, 1800×.
FIG. 7. Marinum group ($2n=14$) (B51).
FIG. 8. Marinum group ($2n=28$) (B1038), obviously not an autotetraploid.
FIG. 9. Murinum group ($2n=14$) (B600), two unlike pairs of sat.-chromosomes.
FIG. 10. Murinum group ($2n=28$) (B1041), note three pairs of sat.-chromosomes, the two from the diploid group are present.

There is a pair of short median and a pair of short submedian chromosomes; and a pair of long median chromosomes. The others are too much alike to be identifiable.

The detailed examination of the chromosomes of the diploid and the re-examination of the tetraploid have confirmed the previous assumption that autopolyploidy is not involved. The tetraploid has one genome corresponding to that of the diploid and one genome from an unknown parent. This genome is marked as having one pair of sat.-chromosomes with a small satellite, and one pair of short median chromosomes.

In some of the cells of the root tip of one plant of B1041 there were extra constrictions in the chromosomes. The most noticeable and consistent secondary constriction was located in the short arm of a sat.-chromosome. If present the constrictions were in two chromosomes, presumably a pair. There were a few additional constrictions in other chromosomes but they were not so consistent and not so obvious. These constrictions are similar to those heterochromatic gaps observable in cold-treated plants of some species, e.g., *Paris* or *Trillium*, and not unlike those emphasized by Mechelke (8) in barley.

Californicum

This species, recently named and adequately described by Covas (2), is similar to several others found growing in North America. Without a careful study it is difficult to separate it from those others having rather long, slender, and upright spikes, in which the laterals are sterile and usually much reduced and the spikelets have short awns but no special distinguishing feature to make classification simpler. The chromosome number of this species is $2n = 14$.

Specimens examined are: B257, received as *H. californicum* from the Herbarium, Davis; B601, received as *H. californicum* Covas from University of California, Berkeley; and one entry, source unknown, labelled as *H. californicum*.

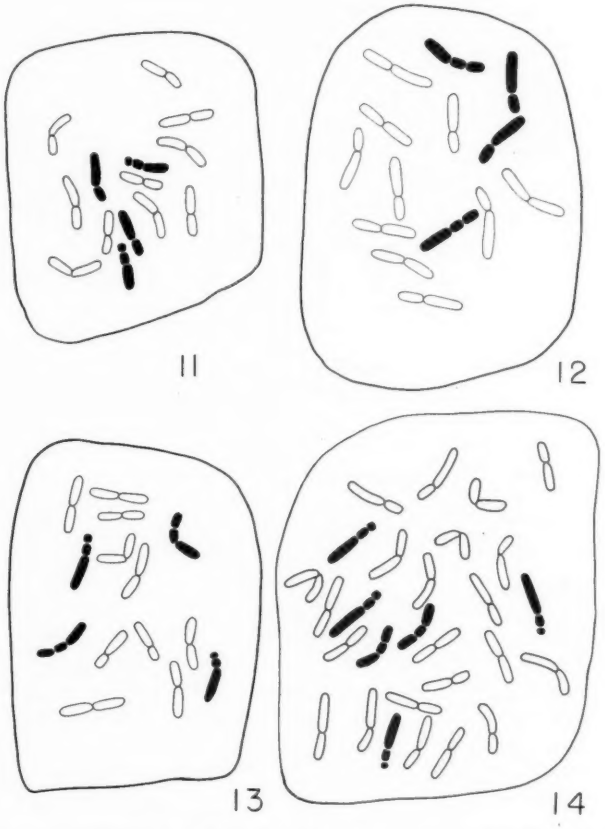
The chromosomes of this diploid species are very much different from those of the species previously described (Figs. 6 and 12). There is one pair of sat.-chromosomes with the satellite longer than the central segment; one obvious pair of long, subterminal chromosomes; and one pair of long median chromosomes. The others are median or submedian with some approaching proportions of being subterminal. With two well-marked chromosomes this diploid set should be traceable if it occurs in combination in some allied allopolyploid.

Stenostachys-pusillum Group

Plants of this group are tall and slender with relatively short leaves. Under greenhouse conditions they are also very late in heading. The spikes are long, up to 10 cm, narrow and somewhat flattened, spikelets are covered with short stiff hairs, lateral florets are much reduced without an awn while the awn of the central floret is short. In B56 the inner glume of the lateral

floret is conspicuously dilated above the base with the other glumes needle-like. In B139 the glumes of the central floret are widened with the inner glume of the laterals much more so. Chromosome number of this group is $2n = 14$.

Only two entries have been established at Ottawa because seed of other collections that have been received did not germinate. They are: B56, received as *H. stenotachys* (*sic*) from Argentina; and B139, received as *H. pusillum* from Brookhaven National Laboratories, New York.



FIGS. 11–14. Drawings of root tip metaphases, chromosomes spaced out in drawing, sat.-chromosomes and other distinctive chromosomes in solid black, 1800×.

FIG. 11. *H. bulbosum* ($2n=14$) (B560), one pair of sat.-chromosomes. In the auto-tetraploid form there are two pairs of sat.-chromosomes.

FIG. 12. *H. californicum* ($2n=14$) (B257), note especially the sat.-chromosomes and chromosomes with subterminal centromeres.

FIG. 13. *H. stenostachys* ($2n=14$) (B56), having two pairs of very unlike sat.-chromosomes.

FIG. 14. *H. jubatum* ($2n=28$) (B420), note three pairs of sat.-chromosomes.

The chromosome complement of this group is shown in Fig. 13. Of the two sat.-chromosomes, one pair is the usual type and the second pair has a long satellite as seen in *H. californicum*. There are two pairs of short chromosomes, one near-median and the second subterminal; one pair of long median and two pairs of long submedian chromosomes. Again the well-marked chromosomes should make it possible to trace this group in combination. The question as to whether one or two distinct species are involved cannot be settled by chromosome morphology.

Bulbosum

In this species group the base of the stem or crown is swollen into a conspicuous bulb. Plants, especially their flowering stalks, grow very tall, over a meter high. Spikes may be 10–12 cm long. All spikelet parts are correspondingly large and seeds may be as long as normal barley seeds. The laterals are almost as large as the central floret and may be fertile; they are without awns. Anthers are large and extrude at anthesis. Chromosome numbers of this group are: $2n = 14$ and $2n = 28$.

Those specimens with 14 chromosomes are: B560, received as *H. bulbosum* from C.S.I.R.O., Canberra, collected in the Mediterranean; B561, received as *H. bulbosum*, collected in Italy, same source as B560. The specimens with 28 chromosomes are: B53, received as *H. bulbosum* from Argentina; B55, received as *H. ithaburense*, same source as B53; B144, received as *H. bulbosum*, from Brookhaven National Laboratories, New York, collected in Germany; B331, received as *H. bulbosum* L. from Botanical Gardens, Moscow.

The chromosomes of the diploid species are shown in Fig. 11. There is only one pair of sat.-chromosomes and one pair of long subterminal to submedian chromosomes; the others are either median or near-median with no conspicuous distinguishing shapes. In the tetraploid species there are two pairs of sat.-chromosomes and two pairs of long subterminal to submedian chromosomes. From the sizes and shapes of the chromosomes it is fairly obvious that the plants are autotetraploids. The presence of quadrivalents at metaphase of meiosis supports this conclusion.

Besides the three special entries listed above, we have 27 other collections of *H. bulbosum* that have not been examined in detail. However, a preliminary examination indicates that most of them have 28 chromosomes. These samples are from different locations in Europe and North Africa. The autotetraploid form of this species is therefore more widespread than the diploid. Under our conditions it is only partially fertile but is quite vigorous vegetatively. Here then is an example of autopolyploidy in *Hordeum* where no change has occurred in the morphology of the chromosomes.

Jubatum

This species, known as foxtail, is a troublesome weed of North America. It is readily recognized by the long-bearded, nodding spikes, and is sometimes grown as an ornamental plant. The brush-like heads may be yellow or purple. There are some variations in awn length and varieties have been

distinguished by this feature. The chromosome number of most entries is $2n = 28$.

Specimens studied are: B204, received as *H. jubatum* var. *caespitosum*, from Botanical Gardens, Coimbra; B420, received as *H. jubatum* from Germany; B540, received as *H. jubatum* from Chelsea Physic Garden; B512, received as *H. jubatum* from Botanical Gardens, Brussels, all through the Botany Unit at Ottawa; B544, received as *H. jubatum* from the University of Warsaw, Poland.

The chromosomes of *H. jubatum* are shown in Fig. 14. There are three pairs of sat.-chromosomes, one pair with the constriction cutting off a large satellite. The other two pairs are also unique in that the short arm subtending the satellite is very short—the chromosomes are subterminal. In some cells when all the satellites do not show, these chromosomes are obvious as subterminal chromosomes. Besides these three well-marked chromosomes there are two other pairs of subterminal chromosomes.

Two abnormalities were observed in *H. jubatum*. One plant, B420-C-1, had a dicentric chromosome in some of the cells of the root tip studied. A plant of B544 had 42 chromosomes. A detailed study has yet to be made. Available in the collection of species are 45 entries, labelled *H. jubatum*. Some of these might have 14 chromosomes. It might then be possible to study the three forms of *H. jubatum*.

Discussion

While there probably has been considerable change in the morphology of the chromosomes since the origin of a species, there is good evidence that most species have chromosomes of a constant size and shape. If this were not so, then major differences would be expected in the samples of the species collected from different localities. The constancy of chromosome morphology thus permits the establishment of a karyotype for the species. Deviations, some probably of a minor nature, are possible but in general they are rare and confined to special forms of the species. In my view the establishment of a karyotype for each species is justifiable.

How does our karyotype analysis compare with the evidence from former studies? It is not possible to compare all the chromosomes as drawn by Chin (1) for *H. bulbosum*, *murinum*, and *gussoneanum*. He found two pairs of sat.-chromosomes in these species and also two in *H. vulgare* and related forms. Besides an extensive report on the chromosomes of *H. vulgare* and the allied group, Oinuma (10) described the karyotype of *pusillum*, *gussoneanum*, and *murinum*. His description of the chromosomes of the vulgare group agrees with the present report but his findings for the other three species do not. There is always the danger, though, that different material is involved because in referring to *H. murinum* he states, "This species looks like the former species *H. gussoneanum*". Our material of *gussoneanum* is quite distinct morphologically from *H. murinum*.

Several distinct karyotypes have been described for some species or species groups. Can we deduce anything about the evolution of the various species from this information? In particular can we explain any relationships with *H. vulgare*? From the standpoint of chromosome morphology, diploids of the murinum complex could seem near relatives of *H. vulgare*; plants of *H. jubatum* would be remote. However, crosses of *H. jubatum* by *H. vulgare* are not difficult to obtain, but those of *H. glaucum* (*stebbinsii*) by *vulgare* have not been reported; we have tried and failed. There are therefore limitations for the use of chromosome morphology in indicating relationships. One reason for the success of crosses with *jubatum* may be that it is a polyploid. From limited results available it seems that when crosses between different species are attempted, more success is achieved when at least one of the parents is a polyploid. The question of chromosome morphology and relationship is therefore not settled for the species that will not cross.

Chromosome morphology has proved a valuable aid in showing that certain species are clearly conspecific, e.g., all species of cultivated or similar barleys and plants within the marinum group. The karyotype analysis has not indicated any definite evolutionary trends but it has set up rather definite groups which should be helpful in future work. At most only two or three chromosome pairs can be identified in any one group but they are usually specific enough to make a firm decision. For example, no difficulty should be experienced in selecting the sat.-chromosome and the subterminal from *H. californicum*. The karyotype of *H. bulbosum* ($2n = 14$) is not very different from that of the marinum diploids but there are definite differences in arm length ratios of the sat.-chromosome and the submedian–subterminal pair. When all specimens in our collection have been studied sufficiently we will have information on at least three more groups. We also will have some information on species crosses and together these results may show a pattern or trend in *Hordeum* which at present is not evident.

One interesting aspect of this study is the occurrence of different chromosome numbers in one species. For example, *H. jubatum* has been reported as having $2n = 14$ chromosomes and in our collection most forms have 28, but one has 42. Another example is the two forms in the marinum group. It can be argued that some of the difficulty stems from incorrect identification of the species but it is not likely that plants of *jubatum* could be incorrectly identified. No one so far has attempted to separate the two forms in the marinum complex. Even so giving a new species name would not change the fact that the plants look very much alike and have different chromosome numbers. This study of the karyotypes has shed some light on the origin or background of some of these troublesome species. Autotetraploidy is clearly indicated in *H. bulbosum*, a fact that is substantiated by studies of meiosis. It is also quite clear that *H. murinum* is an allotetraploid and so are the 28-chromosome types of the marinum group. What is not apparent is the identity of the species contributing the other genomes in these allopolyploids. Further studies may show more instances of different chromosome numbers

within a group: we have another species form with 42 chromosomes. A complete examination of all the plants in our collection seems necessary because only then are we likely to pick up the variations which may help us put together more of the history in *Hordeum*.

Acknowledgment

Particular thanks are extended to Dr. D. G. Hamilton, who initiated the study of *Hordeum* species and who maintained the material for several years. Members of the Barley Unit have since assumed this responsibility. The author gratefully acknowledges the excellent co-operation, advice, and technical assistance given by members of the Barley Unit of the Cereal Crops Division.

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STUDIES OF THE METABOLIC RELATIONS OF ALLANTOIN IN WHEAT[1]

R. M. KRUPKA AND G. H. N. TOWERS

Abstract

Changes in the allantoin and allantoic acid content of germinating seedlings and aging leaves of wheat were followed. The results of experiments on photosynthetic assimilation of $C^{14}O_2$ and the feeding of glycine-C^{14} and glyoxylate-C^{14} indicate that in wheat seedlings the root is probably an important site of allantoin synthesis. Glycine-C^{14} was found to be a better precursor of allantoin-C^{14} than glyoxylate-C^{14}. Allantoin-C^{14} was not synthesized from urea-C^{14}. A scheme for allantoin synthesis which is based on these results is outlined.

Introduction

The results of a previous study of glyoxylic acid in wheat (5) suggested that this acid is a hydrolysis product, and glycine a precursor, of allantoin. The results of further studies of the synthesis of allantoin in wheat are described in this paper.

Materials and Methods

Thatcher wheat seed was germinated as before (5) and whole seedlings or excised leaves were used. The method for the quantitative determination of allantoin and allantoic acid together with chromatographic techniques and methods involving the use of carbon-14 have been described (4, 5). The radioactivity of the plant extracts which had been spotted on the filter paper sheet was determined prior to chromatography by counting the surface of the paper using a Berkeley decimal scaler and a thin end-window Geiger-Müller tube. All counts were taken to a mean error of 2% or better. The chromatograms of the radioactive extracts were radioautographed using Kodak X-ray No-screen film for periods ranging from 3 to 8 weeks, depending partly on the amount of radioactivity spotted at the origin and partly on the expected radioactivity of the compounds of interest, as indicated by experience. Under these conditions regions which registered as little as 1 c.p.m. above background appeared on the radioautographs as distinctly darkened areas relative to the lighter background density of the film. The detection method is, therefore, sensitive to small amounts of radioactivity. The radioactivity of a spot on the chromatogram corresponding to the darkened area of the radioautograph was determined by counting at the surface of the paper. Where a spot was larger than the counter window it was divided off into smaller rectangular areas which were determined separately. The total number of counts in the spot was referred to the radioactivity at the origin as a percentage. The spot was identified by its relative position and by the spraying of duplicate chromatograms with specific reagents. After radioactivity determinations were made, the identity of the spot was confirmed by spraying the radioautographed chromatogram with either ninhydrin or Ehrlich reagent.

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Contribution from the Botany Department, McGill University, Montreal, Que.

Experiments and Results

(*a*) *Changes in the Allantoin and Allantoic Acid Content of Germinating Seedlings*

Seedlings and grains were analyzed quantitatively for total ureides (allantoin and allantoic acid) over a period of 7 days. The results are shown in Table I. The ureide contents of the grains are expressed in relation to the dry weights of the embryos associated with them and not to the dry weights of the grains themselves. In this way they may be compared directly with the values given for the embryos.

TABLE I

Ureide content of wheat embryos and grains during germination

Days after wetting of grain	Ureide* content (μg per g dry weight of embryo)	
	Embryo	Grain
0	Not detectable	Not detectable
2	2500	—
3	400	0
4	1000	530
5	1200	250
6	900	320
7	800	—

*Allantoin plus allantoic acid.

Visual inspection of chromatograms of extracts developed in water-saturated phenol and sprayed with Ehrlich reagent showed that allantoin, but not allantoic acid or urea, was present in 2- and 3-day-old embryos, in both the roots and shoots of 4- and 5-day-old seedlings, and in the shoots of 7-day-old seedlings. The roots of the 7-day-old plants contained detectable allantoic acid in addition to a much larger quantity of allantoin.

(*b*) *Changes in the Ureide Content of Aging Leaves*

Samples of first leaves of wheat grown in soil in the greenhouse were analyzed for total ureides 14 days after planting, when the leaves had reached a stage of early maturity; 21 days after planting, when they were fully mature; and 31 days after planting, when they were wilting. The results are shown in Table II.

TABLE II

Ureide content of first leaves of wheat during aging

Days after planting	Ureide* content (μg per g dry weight)
14	710
21	240
31	90

*Allantoin plus allantoic acid.

(*c*) *Photosynthetic Assimilation of $C^{14}O_2$ by Wheat Seedlings*

(i) Six-day-old green seedlings were placed in a desiccator in which 50 μc of $C^{14}O_2$ was generated and after 30 minutes in the light they were analyzed for 80% ethanol-soluble compounds in the usual way. Glycine was found to be very radioactive, but no radioactivity was detected in allantoin or allantoic acid.

(ii) In a second experiment, 8-day-old entire green seedlings, with their roots submerged in Hoagland's nutrient solution at pH 5.5, were allowed to photosynthesize in the presence of 0.03% $C^{14}O_2$ (100 μc) for 29½ hours. The lid of the desiccator was removed and the plants continued growth in the light for a further 4 days. At the end of this period the roots and shoots were analyzed separately for 80% ethanol-soluble radioactive compounds. Although there was considerable radioactivity in the shoots, allantoic acid, glycine, and glyoxylic acid (determined chromatographically as its 2,4-dinitrophenylhydrazone (4)) were not radioactive. The region of allantoin was very faintly darkened on the radioautographs showing that it was weakly radioactive. In the roots radioactive allantoin was prominent, accounting for 23% of the total radioactivity determined on the chromatogram. A smaller amount of radioactivity (8%) was detected in allantoic acid.

TABLE III

The ureide* content (μg/g dry weight) of degrained etiolated seedlings after feeding with solutions of various compounds

Compound fed†	Ureide* content
Sucrose (0.25%)	870
Sucrose (0.25%) + NH_4NO_3 (0.36%)	1010
Sucrose (0.25% *M*)	780
Sucrose (0.15%) *M*) + glutamic acid (0.1%)	1240
Uric acid (0.006%)	1500
Xanthine (0.25%)	2930

*Allantoin and allantoic acid.
†Duration of feeding 33 hours.

(*d*) *The Effect on the Ureide Content of the Feeding of Various Substances to Entire Embryos*

Four-day-old degrained seedlings were allowed to continue growth in the dark with their roots and scutella submerged in one of a number of solutions. The solutions used were sucrose plus ammonium nitrate, sucrose plus glutamic acid adjusted to pH 6, uric acid, and xanthine. Degrained seedlings in a solution of sucrose were used as a control. The ureide contents were determined after 33 hours. Table III shows these results.

(*e*) *Feeding of Glycine-C^{14}*

(i) Sections of first leaves at a stage of early maturity (from plants grown in soil in the greenhouse) were vacuum infiltrated with water and floated on the surface of a solution of glycine-2-C^{14}, in the presence or absence of 0.36% NH_4NO_3. Feeding was in the light for 24 hours. Analysis of chromatograms of the 80% ethanol-soluble fraction showed that in the feeding with glycine-

2-C^{14} alone, 2.7% of the radioactivity spotted at the origin was in allantoin. In the feeding of glycine-2-C^{14} together with ammonium nitrate the corresponding figure was 3.1%.

(ii) A total of 0.003 mM glycine-2-C^{14} (specific activity 3.2 mc/mM) in 0.01 *M* potassium phosphate buffer, pH 5.2, was fed through the roots to entire 4-day-old green seedlings in the light for 5 hours. The seedlings were washed free of glycine-C^{14} and placed with their roots in tap water and allowed to continue growth in the light for 12 hours. They were then sampled for analysis. In the root extract, approximately 8% of the radioactivity spotted at the origin, which included unchanged glycine-C^{14}, was in allantoin. No radioactivity was detected in allantoin or allantoic acid of any of the shoot extracts.

(iii) This experiment was similar to that described in (*e*) (ii) except that the duration of feeding was shorter (15 minutes) and only the roots were analyzed for radioactive compounds. After being transferred to tap water the roots were sampled immediately and after an additional 15 minutes, 45 minutes, $1\frac{3}{4}$ hours, and $3\frac{3}{4}$ hours. Radioactive allantoin was first detected on radioautographs of extracts taken 2 hours after the feeding commenced.

(*f*) *Feeding of Glyoxylic Acid-C^{14}*

(i) Segments of green leaves from 12-day-old seedlings were vacuum infiltrated with a solution of glyoxylic acid-C^{14} prepared from oxalic acid-C^{14} (6). The specific activity of the glyoxylate was 1.58 mc/mM. After 1 hour the segments were extracted with boiling 80% ethanol. Chromatography followed by radioautography revealed the presence of radioactivity in glycine, glycolic acid, alanine, serine, and two unidentified compounds. No radioactivity was detected in allantoin or allantoic acid.

(ii) The roots of 4-day-old seedlings were immersed in a solution of glyoxylic acid-C^{14} of the same specific activity as in *(f)* (i). After 20 minutes the roots were extracted with boiling 80% ethanol and analyzed in the usual way. Sixty-seven per cent of the radioactivity on the chromatogram was contained in glycine and 33% in glycolic acid. No other radioactive compounds were detected.

(iii) Degrained 7-day-old seedlings were placed with their roots in 5 ml of a solution containing 3.5 μM glyoxylic acid-C^{14} and a similar number was placed with their roots in 5 ml of a solution containing 3.5 μM glyoxylic acid of the same specific activity and 0.2 mM of inactive glycine. The plants were fed in the light for 6 hours, after which they were washed, extracted with boiling 80% ethanol, and chromatographed. From an examination of chromatograms and radioautographs it was found that in the glyoxylate-C^{14} feeding 3% of the radioactivity spotted at the origin was in allantoin and 13% in glycine. In the feeding of glyoxylate-C^{14} with glycine none of the activity was detected in allantoin while 56% was located in glycine.

(*g*) *Feeding of Urea-C^{14} to Wheat Seedlings and to Wheat Leaf Segments*

(i) A total of 0.01 mM urea-C^{14} (specific activity 1 mc/mM) was fed to the roots of 7-day-old degrained seedlings for $4\frac{1}{2}$ hours in the light after which the roots and shoots were analyzed separately for radioactive allantoin.

Radioautographs showed five main radioactive spots with both root and shoot extracts, one of these spots being urea-C^{14}. There was no trace on radioautographs of radioactivity in either allantoic acid or allantoin, although a compound with similar chromatographic properties to allantoin in the solvent systems employed (phenol saturated with water for the first direction and butanol:acetic acid:water::4:1:5 for the second direction) was found to be radioactive.

(ii) Five-day-old etiolated seedlings were fed urea-C^{14} in the same manner as in (i) for 24 hours. Roots and shoots were analyzed separately for radioactivity in allantoin. Although a number of radioactive compounds were detected neither allantoin nor allantoic acid were radioactive.

(iii) Segments of 7-day-old green seedlings (leaves) were vacuum infiltrated with tap water and incubated with urea-C^{14} (0.001 mM) for 3 hours in the dark. No radioactivity was detected in either allantoin or allantoic acid although a number of other compounds were radioactive.

Discussion

Certain indications of the metabolic role of allantoin in wheat emerge from the present experiments.

(i) Allantoin was rapidly formed in germinating wheat embryos but did not appear in the grain in appreciable quantities until 4 days after wetting. This strongly suggests that the initial synthesis of allantoin occurs in the embryo. Allantoin in the grain may have been transported there from the embryo, although this cannot be decided without further study. Earlier studies (4) showed that glyoxylic acid too was undetectable in the dry grain and that during germination the amounts in the grain remained very low. The presence of comparatively large quantities of allantoin and the absence (or presence in very small amounts) of allantoic acid in wheat seedlings may be contrasted with findings in many other plants where allantoic acid predominates (9).

(ii) The decreased quantities of total allantoin plus allantoic acid in leaves during aging suggests that these ureides may be exported to younger regions of the plant or metabolized during this period. Mothes and Engelbrecht (3, 7, 8) have made a detailed study of the physiological importance of allantoin and allantoic acid in maple and in comfrey (*Symphytum officinale*) and conclude that they play the same role as glutamine and asparagine do in many other plants, functioning in the storage and transport of nitrogen.

(iii) The evidence presented here suggests that allantoin is more readily synthesized in the root than in the shoot. (*a*) After photosynthetic assimilation of $C^{14}O_2$ by the leaves of seedlings, radioactive allantoin was plentiful in the roots but scarce in the aerial parts of the plants. (*b*) After the feeding of glycine-C^{14} to seedlings through their roots, radioactive allantoin appeared in the roots but not in the shoots even though considerable radioactivity was detectable in shoot extracts. The feeding described in (*e*) (i) shows that when leaves are infiltrated with glycine-C^{14}, allantoin synthesis does occur, though to a much smaller extent than in roots (*e*) (ii).

(iv) There are two plausible modes of synthesis of allantoin in plants. Allantoin may arise through breakdown of purines, a well-known reaction sequence in animals (2). A second route could involve a reversal of the hydrolysis of allantoic acid. The precursors would then be two molecules of urea and one of glyoxylic acid. There is some evidence for the operation of this pathway in fungi (1).

The results of the present study appear to eliminate a condensation involving glyoxylate and urea to form allantoic acid, and indicate that allantoin is synthesized from glycine, probably by way of purine precursors (Fig. 1). The evidence in support of this may now be reviewed.

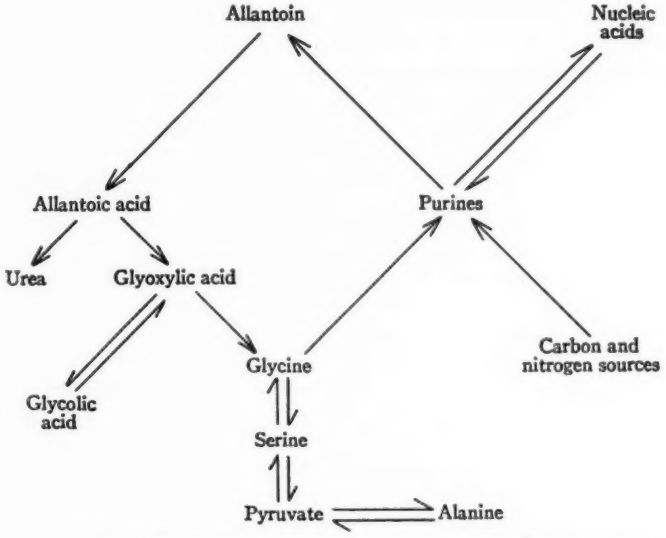

FIG. 1. The metabolism of glyoxylic acid, glycine, and allantoin as suggested by the present studies.

When xanthine or uric acid was fed to the roots of seedlings the level of allantoin in the seedlings increased markedly. Under the experimental conditions, the increase induced by xanthine feeding was three times as great as that with uric acid (276% as compared with 92%), but the concentration of xanthine fed was 40 times as great as that of uric acid. Although the relative rates of penetration into the plant tissue are not known, it is quite possible that on a molar basis uric acid induces larger increases in the allantoin content than does xanthine. Glutamic acid with sucrose resulted in an appreciable rise in the allantoin concentration (72%). This increase was not as great as that due to uric acid, though the concentration of glutamate was much larger than that of uric acid. The presence of ammonium nitrate gave rise to only small increases in the ureide concentration and in the incorporation of carbon-14 into allantoin, showing that its effectiveness is slight. The results are in

accord with the following order for the relative effectiveness of these compounds in inducing allantoin synthesis: uric acid, xanthine, glutamic acid, ammonium nitrate. This order would be expected if allantoin is derived through purine synthesis and breakdown (2).

Glycine-C^{14} fed to roots or leaves was incorporated into allantoin. That glyoxylate is not an intermediate in the conversion of glycine to allantoin has been shown in several ways. The rate of conversion of glycine to glyoxylate in wheat is extremely slow (5) whereas glyoxylate-C^{14} is rapidly converted to glycine-C^{14}. The latter fact could account for the incorporation of C^{14} from glyoxylate into allantoin. It has been shown also that glycine-C^{14} gives rise to allantoin-C^{14} more readily than does glyoxylate-C^{14} (5). When glyoxylate-C^{14} was fed with inactive glycine the latter compound, presumably acting as a metabolic trap, prevented the incorporation of C^{14} into allantoin. There can be no doubt, therefore, that glycine is a more immediate precursor for allantoin synthesis than glyoxylic acid. Finally, it was shown that although urea-C^{14} was readily metabolized, it did not give rise to allantoin-C^{14} or allantoic acid-C^{14}.

Several aspects of the scheme shown in Fig. 1 are noteworthy. Glycine, allantoin, and glyoxylic acid appear as components of an irreversible metabolic cycle, each revolution of which results in the formation of one molecule of glyoxylic acid and one molecule of urea. This pathway may be important in the formation of urea, a compound which has been shown to be readily metabolized in the wheat plant (*g*) (i–iii). The conversion of glyoxylate-C^{14} to glycolate-C^{14} and to glycine-C^{14} (*e*) (ii) has been shown although the relationship between glycolate and glycine cannot be elucidated from the results of the experiments described herein.

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THE POSTGLACIAL DEVELOPMENT OF THE FLORA OF MANITOBA: A DISCUSSION[1]

DORIS LÖVE

Abstract

An attempt is made to establish the development of the flora of Manitoba, Canada, after the complete extermination by the Wisconsin glacier of any pre-existing vegetation.

Based upon geology, palynology, and floristics, a theory is constructed that the ice was at first followed by a cold (marsh) grassland, covering the bottom of the drained Lake Agassiz I, and a riverine spruce (–pine) parkland of western origin, which persisted throughout the Valders period and the damming up of Lake Agassiz II. Around 9000 B.P. a deciduous forest flora started to fill in around the edges of Lake Agassiz II, and a pine–oak savanna occupied the drier portions of the upland. This flora reached its maximum north- and westwards distribution towards the peak of the Hypsithermal. Also during the Hypsithermal it is suggested that a western (–southwestern) prairie flora covered the bottom of the draining Lake Agassiz II, reaching its farthest extension towards the north and east. During the same time, the Arctic flora expanded over the Hudson's Bay Lowland. The last part to be covered by vegetation seems to have been the zone now called taiga, probably as late as 3000–4000 years ago. The spruce forest and its undervegetation seems to have arrived both from the west and from the east, and in recent times (from 2000 to 3000 B.P.) is in a stage of expansion, forcing itself into the deciduous zone, which in turn is expanding over the prairie, save for the checking activities of Man (fire and cultivation).

Introduction

In daily speech Manitoba is often named one of the three Canadian "prairie provinces" together with Saskatchewan and Alberta. Botanically, nothing could be more misleading as a general description of this province, because "prairie," in the true sense of this word, occupies only a fraction of its area, its major portion being covered with forest. Phytogeographically, Manitoba has a most interesting position on the North American map, representing a crossroads of flora elements from both north and south, east and west. In addition to this, the postglacial history of the province as we know it today makes it one of the floristically youngest areas on our continent.

Pre-Wisconsin Conditions

It seems fairly safe to say that whatever flora existed in Manitoba during the third interglacial was entirely wiped out during the fourth glacial period, the so-called Wisconsin Ice Age. Nowhere in this flat province is there any 'ndication of a nunatak area, nor has anything been found that could represent a refugium area. The earliest recovery of the flora in the southern parts of the province may date from somewhat before 11,000 B.P. (B.P. = before present), and the last remnants of the ice may have disappeared from its northlands as late as between 3000 and 4000 years ago.

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Contribution from the Institut Botanique, Université de Montréal, Montreal, Que.

Inside the borders of Manitoba only scanty evidence indicates the nature of its pre-Wisconsin flora. Tyrrell (96) reported on some fossils found at "Rolling River" (now known as "Roaring River") just north of Duck Mountains south of the present Swan River (cf. Fig. 1), which he considered as interglacial relicts. They were found in a 70-ft-thick stratified sand under till and consisted of diatoms, phanerogams, and molluscs (Table I). Altogether the species present in this layer indicate a flora and a fauna similar to what is found in the same area today (Bursa, Wayne, personal communications) and may mean that the climate at that time was similar to the present (Wayne, personal communication).

Penhallow (74), in a review of Pleistocene fossils from Canada, referred to Tyrrell's list but added two more species from Manitoba, *Larix churchbridgensis* Pen. and *Thuja occidentalis* L. The first was discovered by Tyrrell at Churchbridge (due to changes in the provincial areas now just west of the Manitoba–Saskatchewan border; cf. Fig. 1), and later also by Elliot in southern Manitoba (Sect. 23, Township 3, Range II). It is considered by Penhallow (74) as a form somewhat intermediate between "*Larix occidentalis*" and "*L. americana.*" The second species, *Thuja occidentalis*, is most likely a misquote from Dawson (24), who gave "brick clay pits near Montreal" (Dawson (24), p. 422) as his locality. However, in the same paper, Dawson

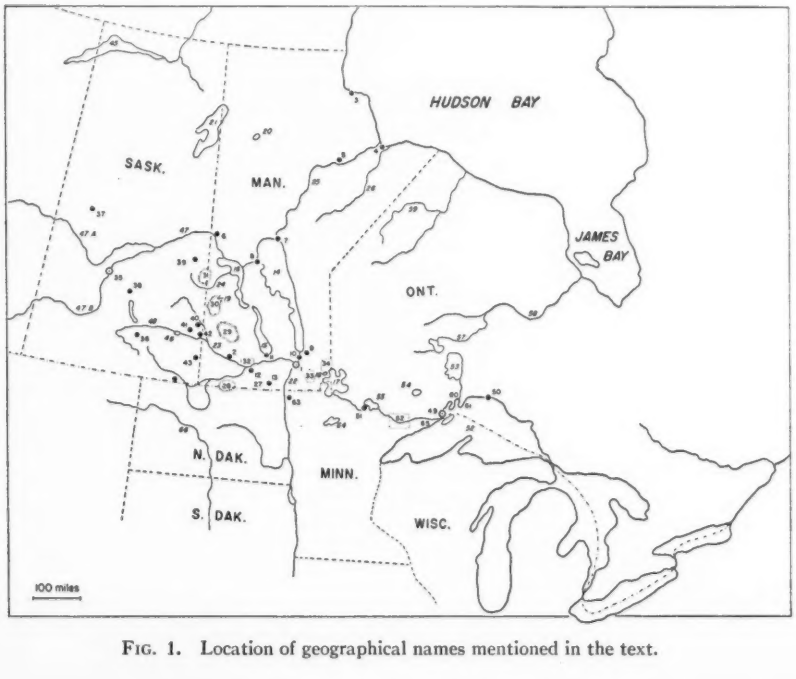


FIG. 1. Location of geographical names mentioned in the text.

mentioned several other collections from "Leda clay, Montreal." This has in Penhallow's paper become "Leda River, Manitoba," a locality unknown in this province. Nothing contradicts *Thuja occidentalis* existing in Manitoba during the last interglacial, but this particular report by Penhallow (74) must be rejected.

A "nest of spruce cones" was found under 90 ft of drift in Turtle Mountains, S. Manitoba (Fig. 1), during well-drilling operations (32). The cones were identified as belonging to *Picea Mariana* by Mr. Gill, Manitoba Provincial Forestry Department, but were unfortunately lost before a carbon date could be made. Elson (personal communication), however, considers them as interglacial fossils, possibly contemporaneous with the above-mentioned *Larix churchbridgensis*, and indicating a post-Sangamon–pre-Wisconsin climatic condition slightly cooler than today.

Enough indications, however, have been found in other Canadian provinces and in various locations of the northern United States to enable us to assume that the vegetation of the last interglacial, at least towards its end, was in general much the same as today with a *coniferous forest* in the western mountains (44, 45, 46) and on the precambrian shield, even reaching south of the present Great Lakes (4, 15, 17, 19, 38, 41, 55, 62, 83, 100), a *deciduous forest* in the eastern United States (9, 10, 11), and a *grassland* on the western plains (85).

FIG. 1 (*Concluded*)

MANITOBA

1. Winnipeg
2. Brandon
3. Fort Churchill
4. York Factory
5. Gillam
6. The Pas
7. Norway House
8. Grand Rapids
9. Brokenhead
10. Lockport
11. Delta
12. Cypress River
13. Morden
14. Lake Winnipeg
15. Lake Manitoba
16. Lake Winnipegosis
17. Lake of the Woods
18. Falcon Lake
19. Singoosh Lake
20. MacBride Lake
21. Reindeer Lake
22. Red River
23. Assiniboine River
24. Swan River
25. Nelson River
26. Hayes River
27. Pembina Hills
28. Turtle Mts.
29. Riding Mts.
30. Duck Mts.
31. Porcupine Mts.
32. Spruce Woods Forest Reserve
33. Sandilands Forest Reserve
34. Whiteshell Forest Reserve

SASKATCHEWAN

35. Saskatoon
36. Regina
37. Meadow Lake
38. Watrous
39. Hudson Bay Jct.
40. Churchbridge
41. Zeneta
42. Welby
43. Moosomin
44. Roche Percée
45. Lake Athabasca
46. Crooked Lake
47. Saskatchewan River
    a. North; *b*. South Branch
48. Qu'appelle River

ONTARIO

49. Port Arthur – Fort William
50. Schreiber
51. Fort Frances–International Falls
52. Lake Superior
53. Lake Nipigon
54. Dog Lake
55. Rainy Lake
56. Rainy River
57. Ogoki River
58. Albany River
59. Sachigo River
60. Thunder Bay District
61. Sibley Peninsula
62. Quetico Park

MINNESOTA

63. Bronson
64. Itasca Park
65. Cooks Co.

NORTH DAKOTA

66. Missouri River

TABLE I

Interglacial fossils from "Rolling" (now = Roaring) River, Manitoba

As listed by Tyrrell, 1890–1891	Modern synonyms
(*a*) Diatoms	
Navicula laca	= *N. lata* Greg. (?)
Encyonema prostrata	= *Cymbella prostrata*
Denticula lauta	(specific name a mistake ?)
Licmophora ?	
Cocconeis	
	(Corrections: A. Bursa, personal communication)
(*b*) Higher plants	
Taxus baccata L.	= *T. canadensis* Willd.
Seeds of a conifer	
Elodea canadensis Michx.	
Vallisneria ?	
	(Corrections: the author)
(*c*) Molluscs	
Lymnaea catascopium ? (Say) var. with short spire	
Valvata tricarinate (Say) plus a keel-less var.	
Amnicola ?*porata* (Say)	= *A. limosa* (Say)
Planorbis bicarinatus (Say)	= *Helisoma anceps* (Menke)
Planorbis ?*parvus* (Say)	= *Gyraulus parvus* (Say)
Pisidium abditum (Haldeman)	= *P. casertanum* (Poli)
Sphaerium striatinum (Lam.)	
	(Corrections: W. J. Wayne, personal communication)

There is also reason to believe that a relatively rich flora existed in the area far north, around the Arctic Archipelago, and that at least parts of this flora survived there throughout the entire Ice Age (47, 48, 56, 58). Another area where a preglacial flora persisted during the entire Ice Age is the large refugium in Alaska–Yukon (47, 48), probably the most important one for the development of at least the western Canadian flora. This refugium may even have been connected by an "oscillating" corridor through northern Alberta to the unglaciated area south of the ice (44, 45, 46).

Proglacial and Full-Glacial Conditions

When the Wisconsin Ice Age began, it seems to have started as an alpine glaciation in the Rocky Mountains, later to be followed by the continental glaciation, the Laurentian ice sheet, with an early accumulation center somewhere around the present Hudson Bay (Labrador–Ungava–Keewatin), i.e. south of the present Arctic Archipelago (cf. Flint (38)). The glaciation of course involved a considerable deterioration of the climate with consequent disturbance of the vegetation. Among the questions that spring to mind when probing these circumstances are the following: How far-reaching was the climatic change? How sudden was it? What chances did the vegetation have to adjust to the changing conditions?

There is plenty of evidence that the cooling affected the world on a practically global scale, so that non-glaciated areas received an increased amount

of rainfall, making regions flourish and cultures arise where we now have desert conditions (38). The cooling also affected marine life in our oceans (33, 95). Many theories have been launched to explain the occurrences and fluctuations of our Ice Ages (52, 73). The initial formation of an ice sheet need not necessarily be caused by a very intense cooling of the climate, but rather may be activated by a moderate cooling and an increase in precipitation. When the ice mass has formed, however, its mere existence must have a marked cooling effect on the climate in its immediate vicinity (38). In this paper we must limit ourselves to the effects rather than causes of the glaciation and state only that the glaciation eventually caused an all-over drop in average temperature.

The fourth continental glaciation in northern Canada seems to have built up over a rather wide area (38), but it must have taken a considerable amount of time for the initial snow and ice mass to accumulate before it developed into a moving body. Ahlmann ((1) p. 9) states that ".... the lag between climatic change and the change in the form of the glacier may be longer than the period of climatic change itself." Further, on the basis of observations by Matthes (63), he says ((1) p. 10) ".... Ice confined in a reservoir remains inert under steadily increasing pressure until a certain point is reached when flow sets in slowly at first, but increasing rapidly in velocity, even though the pressure remains constant or is diminished. The flow then continues with gradually diminishing velocity until the reservoir is well depleted, when the ice-mass returns to its inert stage."

This could mean (though continental ice is not confined to a reservoir) that because ice is a substance which gives and becomes plastic under pressure, an ice mass with sufficient accumulation in one area might move out and spread into another area where it could persist in a climate otherwise unsuitable for the formation and maintenance of glaciers only because of the feeding from behind. Eventually the advance of the glacier is checked by either lack of accumulation (= pressure) or too much loss through evaporation and melting at the edge. A combination of the last two factors eventually leads to the recession of the glacier. These features are easily observed in our present mountain glaciers, e.g., in North America (53), but they were equally important for the development, growth, and recession of continental ice sheets.

The severest climate may thus not have occurred when the ice sheet advanced, but rather after it had consolidated itself, and a considerable improvement may already have taken place before it actually started to retreat.

A *direct* effect on the vegetation in front of the Wisconsin glacier does not seem to have extended much more than ½ to 1 mile ahead of the ice body (15, 64), and is mirrored in the decreasing size of yearly rings in trees killed *in situ* by the advancing glacier. In Minnesota the last 20–25 annual rings of white spruce are very narrow and show a marked retardation of growth in trees that grew at Bronson 36,000 years ago ((83); carbon date, cf. Elson (31)). This is attributed to the approach of the Wisconsin ice sheet, which killed these trees. In Ohio a similar growth retardation was observed in spruce logs over a period of ca. 70 (50–97) years before those trees were crushed under

the ice rim sometime between 24,000 and 16,000 years ago (15). Thanks to such measurements, as well as geological investigations and radiocarbon-dating, it has been possible to estimate the advancing speed of the Wisconsin glacier, though it must have varied during different times and in different areas (38). Flint (38) estimates the average advancing speed at 75 m/year. Goldthwait (41) calculates with an average of 120 m/year (223–481 ft) in northern Ohio and 30 m/year (43–204 ft) in southwestern Ohio. In southern Ohio, along the very rim of the maximal glacial advance, Burns (15) counts 13 m/year (38 ft). On the basis of Rosendahl's (83) observations, it seems that the advancing speed of the glacier in northern Minnesota may have been even faster than any of the above figures indicate.

From the above-mentioned evidence, as well as modern observations by Mathews (64) and Lawrence (53), we have abundant proof that advancing glaciers do overtake living forests before these have a chance to "escape" or "change" due to climatic deterioration in front of the ice (cf. Deevy (26)). In southern Ohio the living trees overrun by the glacier were not only "northern" species like *Picea*, *Larix*, etc. but also such "southern" species as *Quercus*, *Fraxinus*, *Fagus*, and either *Robinia* or *Maclura* (15), indicating that there could not have been too long a lag between a relatively warm climatic period there and the arrival of the glacier. Miss Braun expressed the same feeling when she stated ((9) p. 217) ". . . . There is abundant evidence of persistence of southern forms and communities not far from the ice-front. . . ." and "fossil and vegetational evidence both indicate that the deciduous forest was not displaced south of the glacial boundary except in a band of varying width along the ice-front." (Cf. also Braun (10, 11).) These ideas seem quite plausible if we accept the fact that the ice pushed down into this area mainly because of an immense submarginal accumulation in an otherwise temperate thermal region at its southern border (38). According to Visher (97), Indiana's summers during the glaciation were relatively warm.

However, Miss Braun (9, 10, 11) does not deny that a southwards migration of northern species took place in front of the ice, "invading the established vegetation insofar as suitable habitats were available or weakened competition of more southern forms permitted" ((9) p. 216). This citation is extremely important to keep in mind when we deal with Pleistocene migrations. *Preglacial and postglacial migrations are two very different matters.* In the latter case the plants have a chance of rapidly advancing over a freshly denuded ground, but in the former they must overcome the resistance of an already established vegetation at the same time as they themselves eventually experience a growth retardation due to the deteriorating conditions.

Lawrence (53) has been able to demonstrate that a period of 170 years was necessary after ice melt for a mature Sitka spruce forest to develop at Glacier Bay, Alaska. During the recent warming up of the Arctic, the tree-line in parts of arctic North America has been able to advance 2 miles during the last 30 years (U.S. Weather Bureau, *in* ref. 51). In Germany the postglacial expansion of *Picea Abies* (L.) H. Karst. with a new generation every 30–40

years has been ca. 160–170 m/year, whereas in Sweden, where the life cycle of this species is slower, the advance has been only 70–75 m/year (37).

Today, in a period favorable for the expansion of spruce, the advance of a conifer forest in southern Manitoba is not more than ca. 3 miles/100 years (= 30–40 m/year; Rowe, personal communication). General observations in Canada show that white spruce seed fall to the ground in September, not far from the mother tree, allowing an advance of ca. 200–500 m/50 years (= 1 generation), which would mean less than 10 m/year. Black spruce seeds, released in the winter and able to coast over ice and snow, may enable this species to advance about 2000 m in a similar 50-year period. Jack pine with its serotinous cones often disseminates its seeds by fire (Holst, personal communication). Water dispersal of seeds is considerably faster and more wide-ranging, and as the seeds do not seem to be damaged by it, this may have been the most important mode of dispersal in late-glacial times (84).

The advance of a conifer forest into a broad-leaved one (in Manitoba = aspen zone) may have been the relatively fastest, as the soil there is already somewhat leached and shelter and shade for young growth is available. As mycorrhiza, necessary for some conifer growth, develops not at all or only tardily in a basic soil, the invasion of grassland can be slow, except for white spruce, which seems able to overcome such difficulties (84). Conifers need a minimum of ca. 15–20 years of age (pine 10–20, spruce 15–25) to become seed-bearing if free-standing, longer (30–60 years) if crowded (37), and the colder the climate the longer it takes for the trees to become mature. The cold also affects the quality of the seeds and the period between "seed-years," thus, also the advancing speed of the forest as such. In northern Finland, there was virtually no seed year for pine (*Pinus silvestris* L.) between 1850 and 1910 (1), but there have since been several.

All the above data refer to postglacial migration rates. A *fast*, preglacial migration could, according to my mind, only take place over open areas (as e.g., a denuded riverbank, a coastal shelf being laid bare by receding ocean water, etc.; cf. also Braun (9)), but not into already closed vegetation or into soil conditions normally unfavorable for the fast expansion of a certain flora. It seems, therefore, from a purely biological standpoint, that the chances for a rapid advance of northern forests, able to keep ahead of an ice sheet approaching with a speed of anywhere from ca. 10 m/year (cf. Burns (15): 1 mile/140 years) to 100 m/year (cf. Goldthwait (41): 1 mile/13 years), or maybe even faster (83), must have been very small, if not nil. Rudd (85) is also reasonably sure that no forest grew in front of the maximum expansion of the ice in North Dakota, and that "forest" invaded this state only very lately after the complete disappearance of Lake Agassiz.

The most important periods for the migration of the northern flora must therefore have occurred during times when the ice body halted and remained inert for a while during its general advance. In such intervals the peripheral flora was given a chance to consolidate itself, e.g., so that less hardy species died out because of the proximity of the ice and the resulting severe climatic conditions, giving hardy species a chance to migrate away from the ice into

vacant spaces. This "pulsating" migration may have been more prominent in the early phase of the initial build-up and advance of the continental glacier, but could never have caused the enormous mass migrations over wide distances postulated by Deevey (26). Probably the migrations were limited to very moderate areas, but exceptional water dispersal of some northern species to far south of the glacier, e.g., along drainage channels, seems much more likely.

Therefore I join Hultén (48) in saying that the last glaciation meant far more an extermination than a displacement of flora, and especially so in interior North America. I also dare to propose the following hypotheses, valid especially for the Manitoba area:

(*a*) The Wisconsin ice sheet was not headed by an arctic flora in *the interior* of the North American continent, because the ice developed so as to separate the arctic flora from the area south of it, and because an arctic flora would be unable to migrate easily into an already closed community.

(*b*) The glacier was not preceded by a belt of northern coniferous forest penetrating out onto the western grassland, but this forest was overtaken and exterminated by the ice, which reached right out on the bare grassland.

(*c*) Reserves of northern coniferous forest, mixed forests, and *southern* deciduous forest existed relatively close to the ice border in present central and eastern United States.

(*d*) A reserve of both northern conifers and *northern* deciduous trees existed close to the foot of the Rocky Mountains (possibly throughout the Wisconsin glaciation joined to the refugium in Yukon–Alaska by a fluctuating corridor through Alberta).

Late- and Post-glacial Conditions

Before the deglaciation at last began, the flora around the glacier periphery must have undergone a period of sorting out of less hardy species and a conditioning of hardy ones. The ground laid bare by the retreating ice may have held some permafrost, but this need not have prevented it from quickly being covered by vegetation. *The particular kind of pioneer vegetation in each area must be determined by the availability and aggressiveness of the species closest at hand.* Just as the flora in the Rocky Mountains, on the western plains, and in the eastern United States is different today, so it must have been before, during, and after the Wisconsin glaciation, and plant succession in all these areas must thus be different at all times. It should be recalled that even if a certain type of climate stimulates a certain kind of flora to develop, such a flora can only develop if at least a nucleus of species typical of it is available from the beginning. Therefore, as no arctic species were available in interior North America south of the ice border, no late-glacial "tundra" could develop along the ice rim there. The species listed by Andersen (2) from Michigan do not represent "a late glacial tundra or taiga" (62), but are all widely dispersed boreal species, and represent a spruce parkland as the one found today in southern Manitoba in the so-called "Spruce Woods Forest

Reserve," a *Picea–Eleagnus–Artemisia* association. On the other hand, in New England and east of the Appalachians, a "tundra" with at least some truly arctic species not only could (23, 54, 62) but certainly did follow the retreating ice margin, finally becoming stranded there on mountain tops. It may have come from a coastal refugium.

The Period 11,500–9000 B.P.

The deglaciation in eastern North America had already gone on for a long time and the floristic recovery was well under way before the area that is now Manitoba started to appear from under the ice cover. As it did, complications arose for its invading flora in the form of large ice-dammed lakes in front of the retreating glaciers.

The Mankato – Port Huron ice sheet had flowed over Manitoba from a northwesterly direction, but receded in a northerly and northeasterly direction, first exposing the southwest part of the province, Turtle Mountains being the very first to emerge. In front of the ice, reaching south from Latitude 54° down into North Dakota to the present contact between North and South Dakota with Minnesota, Lake Agassiz I was dammed up. This was the situation around 11,500 years B.P. (Fig. 2). The lake first discharged southwards through the Lake Traverse – River Warren outlet, and the Sheyenne, Elk-valley, Pembina, Assiniboine rivers spilled into this lake against the ice margin (31).

It seems that the only flora available and aggressive enough to invade the newly deglaciated land in Manitoba was that from the bare western grassland (85). Whether this pioneer grassland flora should be named "prairie" or "tundra" or whether neither term should be used for it can be discussed (cf. Martin (62), footnote p. 470). "Prairie" indicates to many a hot, dry condition, and the climate at this time was certainly not so. 'Tundra" seems climatically more acceptable but implies to many, at least to botanists, the presence of arctic species, and such were certainly not available in Manitoba. Supposing that the climate was "cool–dry" and the ground maybe both frozen and wet, I suggest the term "marsh–grassland" as the most appropriate one for our area.

We will probably never be able to demonstrate the exact composition of this "marsh–grassland," as little peat may have been formed and pollen is easily spoiled in marsh conditions. But it seems to me from studying the present distribution of Manitoba species over North America that there is an element in its flora which might represent such a cool "marsh–grassland" condition (cf. also Table II). It could have consisted of various grasses as *Puccinellia*, *Glyceria*, *Poa*, *Festuca*, *Agropyron* s. lat. (i.e., *Elytrigia* and *Roegneria*, not *Agropyrum* s. str.), and *Elymus*, etc., on the relatively drier portions, and *Scolochloa* as well as sedges and rushes in the wetter portions. Herbs accompanying it could have been hardy species as *Pulsatilla*, *Potentilla*. *Astragalus*, and *Oxytropis*, and, in the water, *Potamogeton*, *Ranunculus*, possibly also *Ruppia* and *Najas* (cf. also Moss and Campbell (72); Hansen (45)). An indication

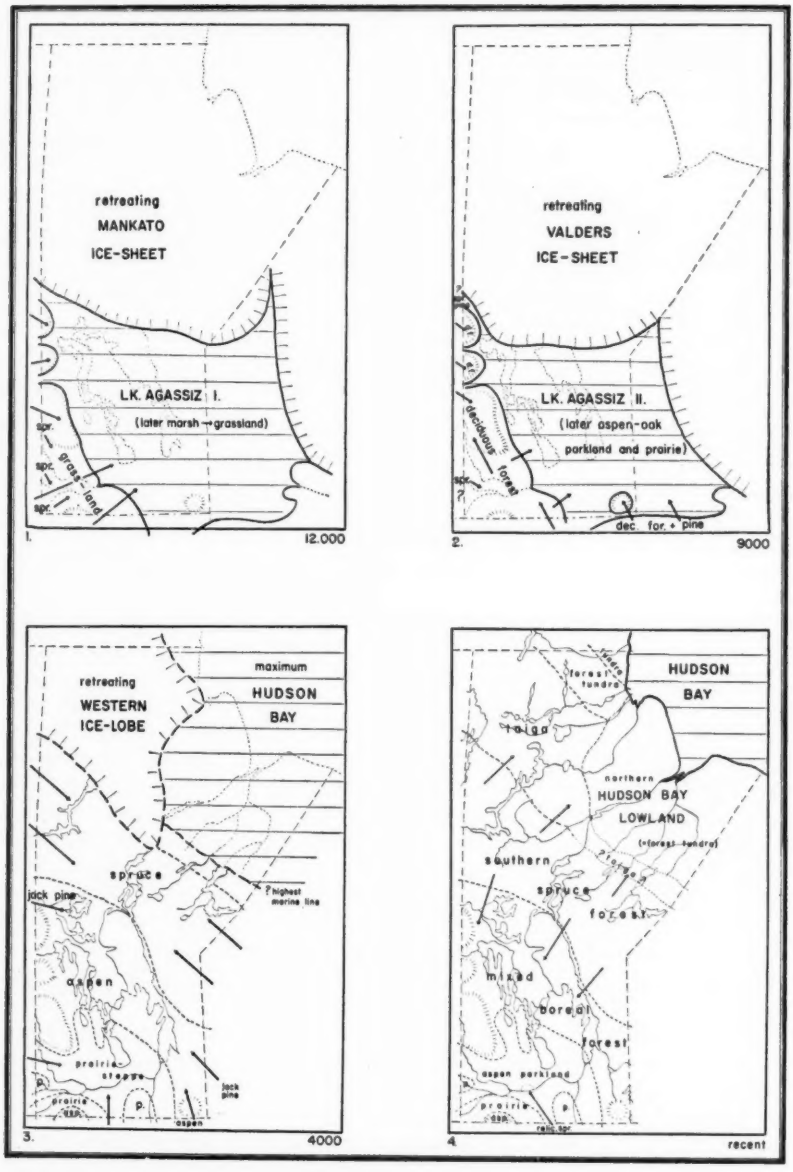


FIG. 2. Schematic representation of the development of the flora of Manitoba from late-glacial to present times. Floristic areas of the present time courtesy of Dr. J. C. Ritchie, University of Manitoba (personal communication).

TABLE II

List of taxa which may represent a "late-glacial marsh–grassland" in Manitoba

To this element belong, in a strict sense:

Monocotyledons

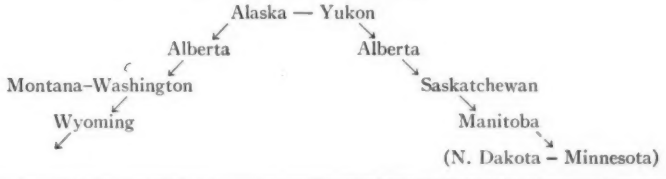
Ruppia occidentalis Wats.
Festuca scabrella Torr.
Puccinellia Nuttalliana (Schult.) Hitchc.
Glyceria pulchella (Torr.) Trin.
Scolochloa festucacea (Willd.) Link.
Elymus Macounii Vasey
Carex Parryana Dew.
Carex atherodes Spreng.
Juncus longistylis Torr.

Dicotyledons

Pulsatilla ludoviciana Heller
Chamaerhodos erecta (L.) Bunge ssp. *Nuttallii* (Pich.) Hultén
Oxytropis splendens Dougl.
Astragalus tenellus Pursh
A. aboriginorum Richardson
A. linearis (Rydb.) A. E. Porsild
Linum Lewisii Pursh
Glaux maritima L. var. *angustifolia* B. Boivin
Gentiana affinis Griseb.
Phlox Hoodii Richards.
Penstemon procerus Dougl.
Symphoricarpus occidentalis Hook.
Erigeron caespitosus Nutt.
E. glabellus Nutt.
Artemisia frigida Willd.

In addition, probably also several others: *Agropyron* s. lat., *Festuca*, *Poa*, *Elymus*, (*Hordeum?*), *Koeleria*, *Trisetum*, etc.

General distribution of the listed species:



NOTE: In Tables II–VII the taxa are, for the sake of convenience, arranged in the same order as in the Flora of Manitoba (88). As the basis for the lists, besides observations by the author, the following literature has been used: Scoggan (86, 87, 88), Ritchie (80, 81, 82), Fraser and Russel (39), Stevens (92), Moore (67), Dutilly, Lepage, and Duman (29, 30), Garton (40, and personal communication), Taylor (93, 94), and Porsild (75).

that a grassland of this type may have followed the ice rim even north of the Agassiz basin is found in northwestern Manitoba near MacBride Lake, where Ritchie (81) found species as *Carex abdita*, *Arabis Holboellii* var. *Collinsii*, *Potentilla pensylvanica* var. *pensylvanica*, *P. arguta*, *Amelanchier alnifolia*, and *Oxytropis splendens* still growing on a high, dry ridge both north and east of their present ranges.

When the ice had withdrawn sufficiently to allow an eastern outlet for Lake Agassiz I, draining it into Lake Superior via a channel over Dog Lake, Ontario (31), such species as mentioned above must have been the plants which invaded the empty lake bottom and followed the drainage channels towards the east. Whether the lake was completely dry or remained essentially as a giant marsh during the ca. 500 years (or less) of interval is not fully known (Elson, personal communication), but it must nevertheless have held wide drainage channels carrying off melt water from the glacier margin as well as dispersing debris and plant parts along its beaches. It may be from this time

that species such as *Oxytropis splendens*, *Artemisia frigida*, and *Festuca scabrella* reached the area around Thunder Bay, Ontario, or at least western Ontario. They are still found there on small "prairie-patches" in completely natural surroundings, to where they could not have been brought by man (40, 93, and Garton, personal communication).

Maybe also a portion of the "arctic" species enumerated by Fernald (36) from the Great Lakes area belong to this time, as e.g., *Botrychium Lunaria*, *Juniperus horizontalis*, *Calamagrostis neglecta*, *Comandra livida*, *Stellaria longipes*, *Parnassia multiseta*, *Geum triflorum*, *Anaphalis margaritaceae* var. *occidentalis*. To call these species "arctic" is very misleading as they are all much more "boreal" in area, and rarely if ever reach up into the true Arctic.

Carbon dates from the Assiniboine Valley in southern Manitoba were made on sandy peat (Y-165, 12,400 ± 240 B.P.) and shell (Y-166, 11,230 ± 480 B.P.) respectively (31). The first of these dates, according to Elson (personal communication), is, however, possibly too old and should definitely be regarded at its youngest level (perhaps even as young as 11,000 B.P. at which age it would fit better to several dates from similar layers in Minnesota). They correspond fairly well with the Two Creeks interval (Fig. 3), a time of spruce minimum, deciduous trees, and pine in New England (25, 27, 54), and of swamp and boglands developing into spruce forests, or beach and marshlands becoming pine forests in Minnesota (cf. Cooper (20); Butters and Abbe (17)). Dates from the beginning of the second lake stage were made on wood (Y-411, 10,500 ± 200 (31)). The drainage channels which in the late-glacial poured melt water into Lake Agassiz from the west (8) were possibly the most important migration routes throughout this period.

What were the possibilities of a forest reaching Manitoba at this time? Contacts with the eastern forest south of the ice could only occur along the southeast margin of Lake Agassiz I (then far south of the Manitoba border), or, after the lake had emptied, over a wide, marshy–boggy, perhaps not even completely drained, lake bottom. There is a fair possibility that especially towards the end of the interval a kind of parkland had been able to form in the Minnesota–Ontario area near Lake Superior (17), but whether it could have penetrated into Manitoba before the climate again deteriorated and Lake Agassiz started to rise due to the advance of the Valders substage of the ice will be hard to say. It seems much more likely, from the composition of the small patches of prairie meadow left in Sandilands Forest Reserve (observation by the author), that the portions of Manitoba which stayed above the second lake level, though surrounded by the water, remained as "grass-islands" till they renewed contact with land. Rudd (85) finds no evidence either of a "forest" in North Dakota from this time, so a southern route seems excluded.

The Assiniboine Valley is, however, on the western side of the lake basin, and trees were growing there already in late-glacial as we know from peat and log samples carbon-dated to around 11,000 B.P. (Y-165, Y-166, Y-411; (31)). A spruce forest, or rather a "park-land", is still growing on the old "Assiniboine delta" in the Spruce Woods Forest Reserve southeast of Brandon (Fig. 1). Its ecology has been studied by Bird (6, 7). A migration to there

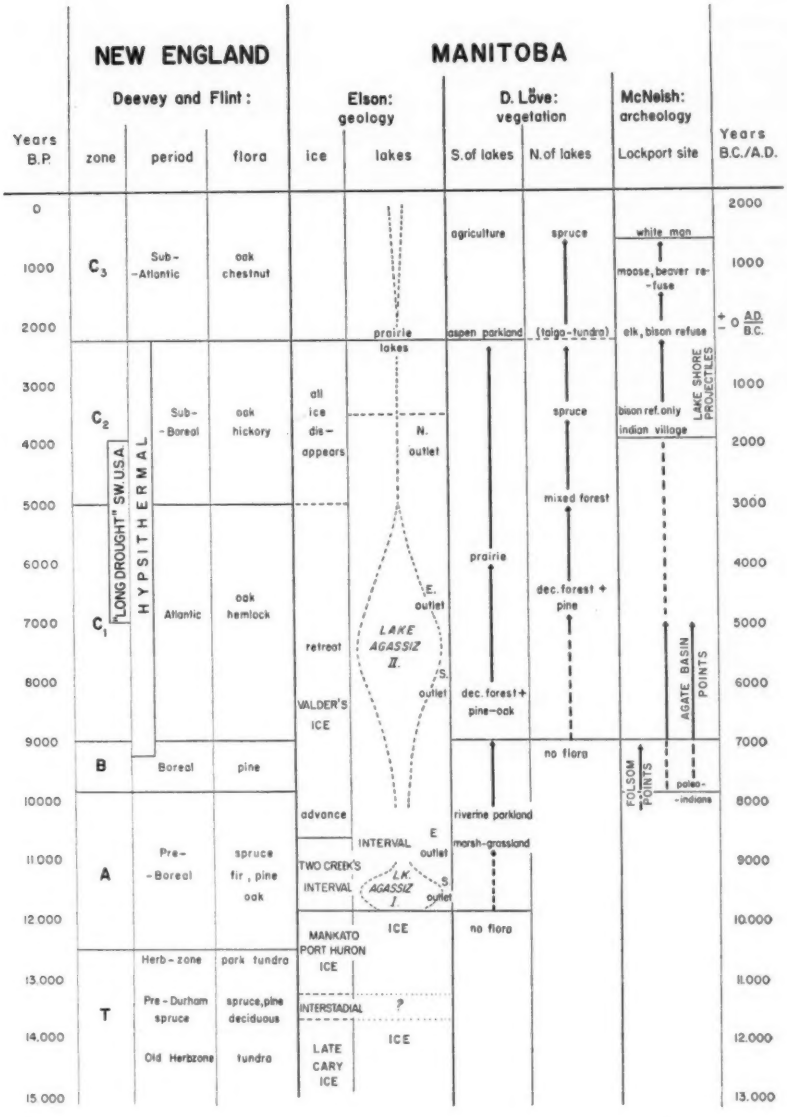


FIG. 3. An attempt to a correlation between postglacial events in Manitoba and New England. Based mainly on Deevey and Flint (28), Elson (31, and personal communication), MacNeish (personal communication), and the theories presented in this paper.

from the east across the marshy lake bottom seems utterly unlikely; a migration around the lake, against prevailing winds, over several wide drainage channels with currents in opposite directions, into a firmly established grassland, seems equally remote (84, 85). But a long-distance migration from a forest reserve in the west along debris-carrying drainage channels, leaving scattered evidence of a riverine spruce forest here and there (cf. Moir (66)), seems much more acceptable. Such excellent support for this kind of migration has been collected and presented by Rowe (84) that it can almost be firmly stated that the early riverine parkland had a western origin. Pollen studies on the peat samples from the Assiniboine Valley (Y-165; (31) and Elson, personal communication) indicate the presence of *Picea glauca* (dominant), *P. mariana*, *Pinus Banksiana* (traces only), *Betula* (some of which is dwarf birch), *Alnus*, *Salix*, and *Populus*. According to Rowe (84) this riverine forest need not be the result of cliseral movement of vegetation zones parallel to the ice front, but an indication that the coulee system offered temporary habitats for survival of a water-dispersed western boreal flora.

Especially, the northfacing slopes of the drainage channels seem to have provided excellent habitats for a northern flora, which in part has been able to survive there till recent time, in spite of changing climate and type of leading trees. For instance, in the Qu'appelle coulee system near Watrous, south of Saskatoon, Rowe (84) lists, among others, the following species in an aspen stand, which we would normally associate with a coniferous forest: *Pyrola asarifolia*, *Chamaenerium angustifolium*, *Chamaepericlymenum canadense*, *Disporum trachycarpum*, *Linnaea borealis* ssp. *americana*, *Viburnum edule*, etc., together with such northwestern grasses as *Agropyrum* (*Elytrigia*) *subsecundum*, *Elymus innovatus*, and *Schizachne purpurascens*. The surrounding area is now open prairie.

In Manitoba these kinds of riverine postglacial forests were likely to occur not only along the Assiniboine River but also along more northern drainage channels north of Riding and Duck Mountains, though the present occurrence of northern flora in these areas may obscure this early element. Shorter coulee systems, not reaching far west, as e.g., the Souris River system, originated on the plains, and do not seem to have carried any traces of forest (independently observed by Rowe (84) and the present authors. Further south in Kidder Co., N. Dakota, a recently discovered "spruce parkland", carbon-dated to 11,480 B.P. (66), may, as I see it, also have arrived from the west along a drainage system (the Missouri?).

If we assume that the Assiniboine delta parkland had a western origin, the question arises whether this locality was indeed the easternmost limit for this dispersal, or if, as in the case of the grassland flora, traces of it could be found further east? Could it be that a reintroduction of some of the famous "Cordilleran element" into the Great Lakes area took place at this time? (See 35, 36, 91.) It might be a likely explanation for the species around the lakes, but would of course not interfere with the same species in eastern Canada and New England, which were undoubtedly separated from their western relatives by the ice sheet (36, 47, 48, 61). "Western types" of spruces are found around

the Lakehead today (Holst, personal communication), though this need not be conclusive evidence. But the similarities between the present Assiniboine delta parkland and the late-glacial forest in Michigan as described in the pollen diagrams by Andersen (2) are more intriguing. His *Picea–Juniperus–Eleagnus–Shepherdia* association with an undervegetation indicating a mixture of grassland (Gramineae, Cyperaceae, *Artemisia*, *Ambrosia*, *Rumex*, and *Pulsatilla*) and boreal species (*Saxifraga*, *Linnaea*, *Polemonium*, and *Botrychium*) much resembles the Assiniboine delta parkland both now and in Two Creeks time. Therefore, to say that the late-glacial Michigan flora and the late-glacial Manitoba flora had a common origin is certainly to go too far, especially as regards the trees, but some of the more aggressive northwestern bushes and ground-cover species might already have been widely dispersed towards the east around the glacier periphery.

In Manitoba, this type of parkland may not have spread much outside the drainage channels and may have lasted as the only type of forest cover till well after the advance and part of the retreat of the Valders substage of the Mankato ice sheet.

The Period 9000–6000 B.P.

At its maximum retreat during the "lake interval" (late Two Creeks – early Valders time), the ice border probably stood north of a line from The Pas, Manitoba, to Sachigo Lake, Ontario; from there down to the west side of Lake Nipigon, hence to its type area in Wisconsin. Around 10,500 B.P. the eastern lake outlets were blocked by ice, and possibly also by crustal uplift; Lake Agassiz II rose and overflowed through the southern outlet at Browns Valley, North Dakota, carving the Campbell strand line along its western shore in Manitoba (31; Figs. 2, 3).

Whereas Lake Agassiz I seems to have been rather short-lived, the second lake lasted for several thousand years, during which the climate shifted considerably. Carbon-dated wood and peat samples from the Assiniboine Valley from this period are 9110 ± 110 and 8020 ± 100 years old (31). It is during the period of the second lake that the first men wandered into Manitoba, leaving first Folsom, later Plainview and Agate Basin, projectiles around the lake shores as evidence of their presence (MacNeish, personal communication; (31)). It seems they were itinerant bands of paleo-indians rather than a settled people, and that they were there between ca. 10,000 and until at least 7000 years B.P. (Fig. 3). This indicates also that even if the winters still may have been severe, the summer climate must have been reasonably tolerable.

Lake Agassiz II seems to have lasted about 4000 years, and existed well into the warm climatic period we usually name the "hypsithermal" (18, 28). In eastern and central United States this lake stage parallelled the A, B, and at least C_1 periods of Deevey (26), i.e., a succession from spruce over pine to deciduous trees (cf. also Deevey and Flint (28)).

The Forest Products Laboratory, Ottawa, has identified the samples of wood collected near Treesbank, Assiniboine Valley, which were dated to 9110 ± 110

B.P. (Y-415, (31), and Elson, personal communication), and found them to consist of *Picea* (*mariana*?) as well as *Ulmus americana* and *Fraxinus* sp. Some details of the wood of the *Fraxinus* indicate it might be *F. nigra*, which is now very rare in the province, but it is not entirely excluded that it represents the more common *F. pennsylvanica* (Elson, personal communication). The sample is nevertheless remarkable, since it is the first indication of "eastern" deciduous tree species on the western side of Lake Agassiz.

Therefore, at ca. 9000 B.P. the climate in the interior seems, in spite of the relative proximity of the ice (150 miles?), to have been warm enough to permit an active upsurge from the south of a broad-leaved forest, which surrounded the southern rim of Lake Agassiz and even in time penetrated the drainage channels leading west all the way into Saskatchewan. Undoubtedly the same climate caused an expansion from the northwest towards east of the "northern" deciduous trees as aspen, poplar, hazel, alder, and birches, but the "southern" arrivals belonged to an oak–maple–basswood–aspen association. Probably they derived from a reserve of this kind between the western grassland and the northeastern coniferous, postglacial forest, having survived not too far south of the ice border (9, 10). It is quite interesting to note here that Deevey (26) sees no objection to a refugium for the tender basswoods in the so-called "driftless area" in Wisconsin (see also Sears (89)), although he otherwise resists the idea of any deciduous flora in the vicinity of the ice border.

In the course of its migration northwards the original maple–basswood–oak–aspen association from the south was gradually thinning out and more tender tree species were left behind but hardy and aggressive ones attained a wider continuous range even than today. Sugar maples reached northeast Minnesota and western Ontario (17, 40), ironwood and large-toothed aspen the southeastern corner of Manitoba, hackberry the southern end of Lake Manitoba, and the basswood the area around Portage la Prairie (88). Elm, ash, and Manitoba maple followed the drainage channels far into Saskatchewan, but the bur oaks came only a little west of the Saskatchewan–Manitoba border (39). The river and lake shores, the shaded escarpments and sandy ridges in the eventually retreating lake proved ideal habitats for this kind of flora (57).

The trees did not, of course, come alone. They were followed by a rich bush and shrub flora as well as many "eastern" herbs. The word "eastern" used here actually describes the modern affinity of this flora to the present deciduous forest in the eastern United States and south of the Great Lakes, although this element arrived in Manitoba from the south and southeast.

At this time the problem of west–east migration seems to become less important for the development of the flora of Manitoba than the south–north migration.

If we analyze the present flora of Manitoba, which comprises ca. 1225 native species (88), and subtract from this number the plants (ca. 575 species) which are so generally distributed over the continent that it is impossible to tell from where they originate, we are left with ca. 650 species which show definite affinities to more distinct distribution areas. Of these a little more

TABLE III

List of taxa, belonging to an "eastern element" from eastern United States and Canada, reaching from southeast and south into Manitoba, occasionally as far west as into Saskatchewan

Monocotyledons

Potamogeton illinoensis Morong
Vallisneria americana Michx.
Bromus Kalmii Gray
Festuca obtusa Biehler
Eragrostis hypnoides (Lam.) BSP.
Sporobolus heterolepis Gray (SE. Sask.)
Sp. neglectus Nash
Leersia oryzoides (L.) Sw.
Zizania aquatica L. var. *interior* Fassett
Sorgastrum nutans (L.) Nash (Sask.?)
Panicum virgatum L. (SE. Sask.)
P. lanuginosum Ell. v. *implicatum* (Scribn) Fern. and v. *septentrionale* Fern.
P. Leibergii (Vasey) Scribn.
P. depauperatum Muhl. (SE. Sask.) v. *psilophyllum* Fern.
P. perlongum Nash
P. linearifolium Scribn. v. *Werneri* (Scribn.) Fern.
P. Wilcoxianum Vasey
P. oligosanthos Schultes v. *Scribnerianum* (Nash) Fern.
P. xanthophysum Gray
Cyperus strigosus L. (south-central Sask.)
C. Houghtonii Torr.
Scirpus Torreyi Olney
S. fluviatilis (Torr.) Gray (north-central Sask.)
Carex rosea Schk.
C. convoluta Mack.
C. alopecoidea Tuckerm. (S. Sask.)
C. normalis Mack.
C. molesta Mack. (Sask.?)
C. Bicknellii Britt.
C. cumulata (Bailey) Mack.
C. muskingumensis Schwein.
C. tetanica Schk.
C. Meadii Dew. (SE. Sask.)
C. Woodii Dew.
C. blanda Dew.
C. granularis Muhl.
C. stricta Lam. v. *elongata* (Böck.) Gl.
C. crinita Lam.
C. intumescens Rudge
Arisaema triphyllum (L.) Schott
Juncus canadensis J. Gay
Luzula acuminata Raf. (central Sask.)
Allium tricoccum Ait.
Smilax lasioneura Hook.
Polygonatum canaliculatum (Muhl.) Pursh
Uvularia sessilifolia L.
Streptopus roseus Michx. v. *perspectus* Fassett
Trillium cernuum L. (Sask.?)
Smilacina racemosa (L.) Desf.
Cypripedium arietinum R. Br. (north-central Sask.)
C. pubescens Willd.
C. candidum Muhl.

Dicotyledons

Populus deltoides Marsh
P. grandidentata Michx.
Salix tristis Ait.
Corylus americanus Walt. (Sask.?)
Ostrya virginiana (Mill.) Koch
Quercus macrocarpa Michx. (E. Sask.)
Celtis pumila Pursh
Ulmus americana L. (E. Sask.)
Laportea canadensis (L.) Wedd.
Asarum canadense L.
Rumex orbiculatus Gray
Tracaulon sagittatum (L.) Small
Bilderdykia cilinodis (Michx.) Greene (north-central Sask.)
Polygonum scandens L.
P. erectum L. (Sask.?)
Persicaria coccinea Greene ssp. *pratincola* (Greene) L. & L.
P. punctatum Small v. *confertiflorum* Meisn.
Chenopodium strictum Roth. v. *glaucophyllum* (Aell.) Wahl
Acnida tamariscina (Nutt.) Wood
Clematis virginiana L.
Myosurus minimus L. (S. Sask.)
Aquilegia canadensis L. v. *eminens* (Greene) B. Boivin (SE. Sask.)
Delphinium virescens Nutt.
Hepatica americana (DC.) Ker.
Anemone quinquefolia L. v. *interior* Fern. (E. Sask.)
A. virginiana L.
Ranunculus fascicularis Muhl.
Caulophyllum thalictroides (L.) Michx.
Menispermum canadense L.
Sanguinaria canadensis L.
Adlumia fungosa (Ait.) Greene
Polanisia graveolens Raf. (S. Sask.)
Saxifraga virginiensis Michx.
Penthorum sedoides L.
Agrimonia gryposepala Wallr.
Prunus nigra Ait.
P. pumila L. (E. Sask., as *P. Besseyi*)
Crataegus punctata Jacq. (S. Sask.)
Cr. succulenta Link. v. *occidentalis* (Britt.) Palmer (SE. Sask.)
Amelanchier humilis Wieg.
Sorbus americana Marsh
Astragalus neglectus (T. & G.) Sheldon
Desmodium canadense (L.) DC.
Amphicarpa bracteata (L.) Fern. (S. Sask.)
Linum sulcatum Riddell (S. Sask.)
Polygala paucifolia Willd. (central Sask.)
P. verticillata L. v. *isocycla* Fern.
Euphorbia Geyeri Engelm.

TABLE III (*Concluded*)

Callitriche heterophylla Pursh
Rhus glabra L.
Celastrus scandens L.
Ceanothus ovatus Desf.
Parthenocissus inserta (Kerner) K. Fritsch
Vitis riparia Michx.
Tilia americana L.
Hypericum virginicum L. v. *Fraseri* (Spach) Fern.
Helianthemum Bicknellii Fern.
Elatine americana (Pursh) Arn. (S. Sask.)
Viola sororia Willd.
V. pubescens Ait.
V. blanda Willd.
V. pensylvanica Michx. v. *leiocarpa* (Fern. & Wieg.) Fern.
V. conspersa Rchb.
Oenothera fruticosa L. v. *linearis* (Michx.) S. Wats.
Aralia racemosa L.
Osmorrhiza longistylis (Torr.) DC. (S. Sask.)
O. Claytonii (Michx.) Clarke
Zizia aurea (L.) W. D. J. Koch (Sask.?)
Cryptotaenia canadensis (L.) DC.
Thaspium barbinode (Michx.) Nutt.
Cornus alternifolia L.
C. racemosa Lam.
C. rugosa Lam.
Pyrola rotundifolia L. v. *americana* (Sw.) Fern.
Dodecatheon Meadia L.
Lysimachia terrestris (L.) BSP.
Steironema hybrida (Michx.) Raf.
St. quadriflora (Sims) Hitchc.
Fraxinus nigra Marsh
Fr. pennsylvanica Marsh v. *Austini* Fern. (E. Sask.)
v. *subintegerrima* (Vahl.) Fern. (E. Sask.)
Gentiana crinita Fröl. (E. Sask.)
G. puberula Michx. (Sask.?)
G. Andrewsii Griseb. (E. Sask.)
G. flavida Gray
Apocynum sibiricum Jacq. v. *cordigerum* (Greene) Fern. (E. Sask.)
Asclepias syriaca L. (Sask.?)
Cuscuta Gronowii Willd. (S. Sask.)
Phlox pilosa L. v. *fulgida* Wherry
Hydrophyllum virginianum L.
Onosmodium hispidissimum Mack.
Dracocephalum virginianum L.
Stachys tenuifolia Willd.
Chamaesaracha grandiflora (Hook.) Fern.
Physalis pruinosa L.
Ph. lanceolata Michx.
Chelone glabra L. v. *linifolia* Coleman
Mimulus ringens L. (E. Sask.)
Mimulus glabratus HBK. v. *Fremontii* (Benth.) Grant
Veronicastrum virginicum (L.) Farw.
Gerardia paupercula (Gray) Britt.
G. aspera Dougl.
Castilleja coccinea (L.) Spreng. (E. Sask.)
Pedicularis lanceolata Michx.
Ped. canadensis L.
Phryma leptostachya L.
Houstonia longifolia Gaertn. (S. Sask.)
Sambucus canadensis L.
Viburnum Lentago L. (SE. Sask.)
V. Rafinesquianum Schultes (Sask.?)
Echinocystis lobata (Michx.) T. & G. (S. Sask.)
Campanula aparinoides Pursh (central Sask.)
Lobelia silphitica L. v. *ludoviciana* A. DC.
Lobelia spicata Lam. v. *hirtella* Gray (S. Sask.)
v. *campanulata* McVaugh
Vernonia fasciculata Michx. v. *corymbosa* (Schwein.) Schub.
Eupatorium perfoliatum L.
Solidago Riddellii Frank.
Boltonia asteroides (L.) L'Her. v. *recognita* (Fern. & Grisc.) Cronq. (E. Sask.)
Aster macrophyllus L.
A. sagittifolius Wedemeyer
A. ptarmicoides (Nees.) T. & G.
A. novae-angliae L. (S. Sask.)
A. cordifolius L.
A. sericeus Vent.
A. lateriflorus (L.) Britt.
A. praealtus Poir.
Antennaria plantaginifolia (L.) Hook.
A. munda Fern. (SW. Sask.)
Rudbeckia laciniata L.
Helianthus tuberosus L. v. *subcanescens* Gray
Cirsium muticum Michx.
C. discolor (Muhl.) Spreng.
Krigia biflora (Walt.) Blake
Lactuca canadensis L. (Sask.?)

than a third, ca. 185 species, belong to a deciduous forest element which reaches Manitoba, but diminishes constantly as it proceeds westwards (cf. Table III; Fig. 4*e*). Only ca. 50 of the 185 are to be found in Saskatchewan, and most of them only in the eastern part of that province (cf. Fraser and Russell (39)). The same tendency is visible in North Dakota, where 110 of the 185 Manitoba species occur, and almost all of them are confined to areas which were covered or bordered by Lake Agassiz (85, 92). In northern Minnesota the same eastern element is of course larger than in Manitoba, but mainly

found in a narrow belt between the prairie and the more easterly spruce–pine forest (34, 67). Small stands of deciduous trees are, however, found here and there inside the coniferous forest, usually in areas bordering or previously covered by Lake Agassiz (12, 13, 14). In the vicinity of and along the north shore of Lake Superior, both in Minnesota (17) and in western Ontario, especially in the Thunder Bay area, we also find such "relic" patches of deciduous forest (20).

There are thus indications that the "eastern" deciduous forest dispersed over an area even larger than today both north- and west-wards. Much evidence can be based on the present distribution of herbs, now on a prairie or in a mixed forest, but normally associated with a deciduous forest. Some noteworthy members, other than trees, of the "eastern element" include, e.g., climbers, bushes, and herbs such as: *Smilax lasioneura* (Riding Mts., Man.), *Menispermum canadense* (Riding Mts.), *Celastrus scandens* (Delta, Man.), *Echinocystis lobata* (E. Sask.); *Corylus americana* (Riding Mts.), *Prunus americana* (Roche Percée, Sask.), *P. pumila* (Hudson Bay Jct. and Welby, Sask.; Sibley Peninsula, Ont.), *Crataegus succulenta* (E. Sask.), *C. punctata* (E. Sask.), *Cornus alternifolia* (Riding Mts.), *C. racemosa* (Pembina hills, Man.; Turtle Mts., N. Dak.), *Acer spicatum* (E. Sask.; Sibley Peninsula, Ont.), *Viburnum Lentago* (SE. Sask.), *V. Rafinesquianum* (Riding Mts.); *Panicum depauperatum* (Moosomin, Sask.), *Cyperus strigosus* (Watrous, Sask.), *Scirpus fluviatilis* (Lac La Ronge, Sask.), *Luzula acuminata* (Meadow Lake, Sask.), *Trillium cernuum* (E. Sask.), *Asarum canadense* (Sibley Peninsula, Ont.), *Aquilegia canadensis* (Crooked Lake, Sask.), *Anemone quinquefolia* v. *interius* (Somme, Sask.), *Amphicarpa bracteata* (E. Sask.), *Polygala paucifolia* (E. Sask.), *Viola pensylvanica* v. *eriocarpa* (Pembina hills, Man.; Sibley Peninsula, Ont.), *Osmorhiza longistylis* (E. Sask.), *Gentiana Andrewsii* (Zeneta, Sask.), *Mimulus ringens* (Hudson Bay Jct., Sask.), *Rudbeckia laciniata* (Riding Mts.), etc.

(The list is based upon Taylor (93, 94); Rowe (84); Garton (40); Scoggan (88); and the author's own observations. Place names (Fig. 1) indicate disjunct localities outside present continuous range.)

In Saskatchewan the "eastern element" met and mixed with a "northern deciduous forest," bringing with it an undervegetation of herbaceous species and shrubs which now reach as far east as Manitoba and western Ontario, e.g.: *Amelanchier alnifolia*, *Symphoricarpus occidentalis*, and *Viola rugulosa*.

In western Minnesota some pollen diagrams from Itasca Park made by Potzger (76) indicate a rather sudden change from a spruce–fir to a pine–oak association at the beginning of what must have been a warmer period, though Potzger does not date it. The diagrams also show a rather high incidence of elm and an occasional occurrence of ash. That trees like aspen and maple are missing from the diagrams may be due to the difficulties in recognizing or finding these pollen types during this less well equipped early phase of American palynology. The presence of both deciduous trees and pines may be taken to indicate that *at the same time* as the deciduous trees advanced in

moist and humid habitats, pines and oaks expanded on drier habitats, creating a sort of pine–oak savanna, or on the driest sites a pine parkland.

Near Brokenhead, ca. 20 miles south of Lake Winnipeg, there is today found a community which may resemble such an early pine–oak parkland: a *Pinus Banksiana – Quercus macrocarpa* savanna with some *Populus tremuloides* and *Alnus crispa*, and an undervegetation consisting of such species as *Hudsonia tomentosa, Lechea intermedia, Gaultheria procumbens, Prunus virginiana, Amelanchier alnifolia*, and grasses among which a very tall growing *Elymus canadensis* is conspicuous. *Cyperus Houghtonii* is found there in one of its westernmost localities, and *Selaginella rupestris* is quite common. A rather good quantity of "eastern" species are thus represented along with the trees, though unfortunately a thorough analysis of the site was never made.

A more typical "jack-pine phase of the mixed forest" is shown by Butters and Abbe (17) from Cooks Co., Minnesota. Dominating tree: *Pinus Banksiana;* associated trees: *Abies balsamea, Picea mariana, Betula papyrifera;* other species: *Amelanchier humilis* (E), *Apocynum androsaemifolium, Aralia nudicaulis, Aster macrophyllus* (E), *Chimaphila umbellata* v. *cisatlantica* (E), *Clintonia borealis* (E), *Cornus canadensis, Corylus cornuta, Danthonia spicatum* v. *pinetorum, Fragaria virginiana, Goodyera repens, Halenia deflexa, Linnaea borealis* ssp. *americana, Lonicera oblongifolia* (E), *Melampyrum linearis, Moneses uniflora, Pteridium aquilinum* v. *latiusculum* (E), *Pyrola secunda, Spiranthes gracilis* (E), *Streptopus roseus* v. *longipes* (E), *Vaccinium myrtilloides*. (Species with an eastern affinity have been marked with (E).)

These two communities in southeastern Manitoba and northern Minnesota are very typical of the pine associations on the east side of the prairie lakes. In addition to *Pinus Banksiana* in southeastern Manitoba, there is also some *Pinus Strobus* and *P. resinosa*. The former reaches north to the Lake-of-the-Woods, the latter has also a disjunct site on Hecla Island in Lake Winnipeg, possibly indicating a previously wider distribution than now.

There is no doubt that the pines on the eastern side of the Lake Agassiz basin originated from the east, where we find a concentration of *Pinus* west of the Great Lakes (cf. also Wilson (101)). Halliday and Brown (43) were of the opinion that all pines in central Canada originated from this center and dispersed east and west. Pines in the Maritimes might have their own disjunct dispersal center, though.

It seems, however, that if the jack pines migrated westwards from the area around the Great Lakes, they ought to have brought along some "eastern" undervegetation, but studying jack pine sites on the western side of the prairie lakes, the undervegetation has a much more marked western nature with only few "eastern" species present. It seems, also, that the jack pine concentration in western Manitoba may be denser than previously assumed (81).

Two sites, quoted from Rowe (84), may serve as examples:

(1) Singoosh Lake, Duck Mt., Manitoba. Dominant tree: *Pinus Banksiana;* other species in order of prevalence: very abundant: *Elymus innovatus* (W); abundant: *Fragaria virginiana, Aralia nudicaulis;* common: *Actaea rubra,*

Anemone riparia (E), *Agastache Foeniculum* (W), *Lilium umbellatum* (W), *Sanicula marilandica*, *Thalictrum venulosum* (W), *Schizachne purpurascens*, *Solidago hispida* (E), *Oryzopsis asperifolia*, *Chamaenerion angustifolium*, *Lathyrus ochroleucus*, *Prenanthes alba*, *Galium septentrionale*, *Petasites palmatus*, *Cornus canadensis*, *Linnaea borealis* ssp. *americana*, *Maianthemum canadensis* ssp. *interius* (W); occasional: *Vaccinium myrtilloides*, *Amelanchier alnifolia* (W), *Hieracium canadense*, *Lathyrus venosus* v. *intonsus*, *Achillea sibirica* (W), *Corylus cornuta*, *Shepherdia canadensis* (W), *Diervilla Lonicera* (E), *Alnus crispa*.

(2) Eastern Saskatchewan, in the southern boreal forest belt. Dominant tree: *Pinus Banksiana;* constants: *Arctostaphylos Uva-ursi*, *Maianthemum canadensis* ssp. *interius*, *Festuca saximontana* (W), *Oryzopsis pungens;* other species in order of importance: *Fragaria virginiana*, *Vaccinium myrtilloides*, *Lycopodium complanatum*, *Sibbaldiopsis tridentata*, *Galium septentrionale*, *Solidago hispida* (E), *Solidago nemoralis* v. *decemflora* (W), *Elymus innovatus* (W), *Arabis lyrata* v. *kamchatica* (W), *Aster laevis*, *Comandra Richardsiana*, *Lathyrus ochroleucus*, *Melampyrum lineare*, *Artemisia caudata* (W), *Rosa* sp., *Antennaria campestris* (W), *Hieracium canadense*, *Vaccinium Vitis-Idaea* ssp. *minor*, *Linnaea borealis* ssp. *americana*, *Arceuthobium americanum* (W), *Pulsatilla ludoviciana* (W), *Carex foena* (W), *Selaginella densa* (W), *Spiranthes gracilis* (E). ((E) = "eastern"; (W) = "western" affinity.)

It is not difficult to see that the two "western" sites are very different in composition from the two "eastern" ones.

That jack pines should have migrated around the southern end of the Agassiz basin without leaving any trace of their route in North Dakota (85) nor in SW. Manitoba, where many localities could have been suitable for relic habitats, seems highly unlikely, especially as deciduous trees following this route left plenty of evidence, and still exist along it. There is, however, a soil zone from Cypress River, southern Manitoba, which has been carbon-dated (on charcoal fragments) to an age of around 2800 B.P. (Elson, personal communication) and which locally contained some peat that probably is the same age. The sample came from an alluvial fan in a very shallow stream, and was badly decomposed. H. G. Ignatius made a tentative identification of the very few pollen grains which could be found in the sample, and saw some pollen of oaks, willow (poplar?), grasses, sedges, *Artemisia*, *Chenopodium*, *Typha*, an Ericaceae, and "a grain or two of a small pine," *Pinus* sp. (*Banksiana?*) (Elson, personal communication). With the exception of these pine grains, the rest of the pollen indicates a flora which is now found in this locality (it has been visited by the present author) and could easily have been the same 2800 years ago. That *Pinus Banksiana* should have existed there at that time seems, however, extremely unlikely and the possibility that the grains could have been wind-borne, rebedded, or the result of other abnormalities is not excluded. Both Elson and the present author are of the opinion that this find should be regarded with suspicion.

A migration across the lake seems also impossible, as jack pines definitely avoid marshy conditions, and a migration route north of the lake was not

open till such a late date—after the complete drainage of Lake Agassiz II, not earlier than 7000 B.P.—that it seems unlikely for the pines to reach all the way from the Great Lakes to the Yukon in the short time available (cf. also Rowe (84)). Jack pines did, however, survive in the Yukon refugium (70, 78) and were found in the "corridor" in Alberta (44, 45, 46), which definitely remained open from the Two Creeks time or even earlier (Elson, personal communication). Quite recently an investigation of organic deposits at Herbert, Saskatchewan (ca. 114 miles west of Regina), has revealed the existence of *Pinus Banksiana* at a level carbon-dated to 10,500 ± 300 B.P. (Kupsch, personal communication). The site, now open prairie, had then a flora similar to the one in the Spruce Woods Reserve, south of Brandon (p. 558), consisting of an open parkland with white and black spruce, jack pine and another pine (not identified), birch, willows, as well as *Artemisia* and *Shepherdia* (Kupsch, personal communication). It seems, therefore, logical that most of the jack pines in western Manitoba should have originated from the west rather than from the Great Lakes center, especially as the trend of migration in general is from west to east, and not the reverse.

A problem that has to be considered, however, is whether the jack pines were able to survive the crowding in a "corridor" with lodgepole pines without completely mixing with this species, or at least without showing strong influence from such a mixture, e.g., in the form of extensive "introgression" of characters. Hybridization between jack and lodgepole pines occurs frequently in the present "overlap area" (70), and results in trees which are more or less intermediate in appearance (65, 70) but cases are also known where the trees have the outer appearance of true lodgepole pine but have turpentines typical of jack pine (65). The jack pine turpentines (pinenes) seem to be dominant over those of lodgepole pines (phellandrenes), which makes it hard, if not impossible, to tell how far such an integrated character can have penetrated east in the jack pine area. The straight-coned character (70) does not seem to be a definite indicator of lodgepole genes, as straight-coned forms of true *Pinus Banksiana* occur both in the Great Lakes area and further east (Quebec and New Brunswick; Holst, personal communication) to where it could hardly have spread in postglacial time. On strictly taxonomical grounds it is thus very difficult to prove that the jack pines had eastern, central, as well as western redistribution centra, but logically, especially with the aid of floristic and ecological studies, I do not see much objection to this hypothesis.

It may be worthwhile to repeat here that traces of (jack?) pine pollen were found in the peat samples from the Assiniboine Valley some 11,000 years B.P. (31, and Elson, personal communication) in an association which we already have decided had a western origin.

The Period 6000–2000 B.P.

The climate from ca. 9000 B.P., when the first deciduous trees and pines immigrated from the south, was becoming increasingly warmer. Retreat of

the Valders ice border opened eastern outlets for Lake Agassiz II north of the Dog River spillway, through the Kaiashk – Pillar Lake – Black Sturgeon channel, and at last through the Pikitigushi – Lake Nipigon into Lake Superior. Series of strand lines lower than the Campbell illustrate these phases. When residual ice in the Keewatin district was severed from the retreating Laurentide lobe by melting into Hudson Bay (not before 7000), an outlet north of the Sachigo Moraine permitted the lake to drain rapidly northwards. Later, but prior to 3600 B.P., the Nelson River functioned as a northern outlet of the basin (31).

After 3600 B.P. there was an Indian settlement at Lockport in the Red River Valley ca. 17 miles north of Winnipeg (Fig. 1). In the bottom layers of the excavations made on this site are found Lake-shore (Signal Butte, Iowa) projectiles regarded as around 3500 years old (Fig. 3), and together with them refuse of bison and other prairie animals (31; MacNeish, personal communication), which indicates that the lake bottom had transformed into a prairie some time between 7000 and 4000 years ago (Fig. 3). This coincides with the middle and upper end of the hypsithermal period (18, 28), sometimes called the "Long Drought," when the temperature rose even higher than today. The conditions must therefore have been ideal for an advance from the west and the south of a heat-loving prairie flora, especially over the drying lake bottom and along the drainage channels, at the same time as further north the coniferous trees spread over the precambrian shield and the deciduous trees reached their northernmost expansion as a continuous forest (Fig. 2), which was further north than today (71).

If we again consider the ca. 650 native Manitoba species, which show some sort of affinity to distinct distribution areas, we find that about half of them, ca. 320, have western affinities, and of these ca. 230 belong to an element associated with the western or southwestern plains (cf. Table IV; Fig. 4*d*). Some, ca. 170 species, seem to have come almost directly from the west via Saskatchewan and western North Dakota, while others, ca. 60 species, appear to have followed the Red River Valley northwards. Grasses like *Stipa*, *Andropogon*, and *Sorgastrum* covered the "meadow-prairie," whereas *Bouteloua*, *Distichlis*, and *Buchloe* filled saline and dry habitats, and *Spartina* and *Beckmannia* the sloughs and stagnant pools. The southern species did not crowd out the already existing "northern grassland" but mixed freely with it, more so in the southern than in the northern part of our area.

With the prairie followed a wealth of herbs, mainly from Polygonaceae, Chenopodiaceae, Rosaceae, Leguminoseae, and Compositae. Among the more extreme heat- and drought-requiring plant families arriving at this date were Cactaceae, represented in Manitoba by three species. If an additional example should be mentioned from the animal world, turtles seem to have come along during the warm period. Two are still found in Manitoba, the snapping turtle (*Chelydra serpentina serpentina*) and the western painted (*Chrysemys picta bellii*).

The cacti and the turtles may serve as indicators that the prairie element arrived fairly early, probably when the eastwards drainage channels were

TABLE IV

List of taxa belonging to a "western element" reaching from western Canada and western and southwestern United States from west, southwest, and occasionally south (via the Red River Valley) into Manitoba and rarely as far east as to western Ontario (Quetico), the Great Lakes (Thunder Bay), or James Bay

Monocotyledons

Potamogeton foliosus Raf. (Thunder Bay)
Alisma gramineum Gmel.
Bromus pumpellianus Scribn.
B. Porteri (Coult.) Nash
Poa arida Vasey
Poa glaucifolia Scribn. & Wiil.
Poa Cusickii Vasey
Catabrosa aquatica (L.) BP. v. *uniflora* Gray (James Bay)
Distichlis stricta (Torr.) Gray
Agropyron trachycaulon (Link.) Malte (incl. v. *majus* (Vasey) Fern.) (syn.: *Roegneria pauciflora* (Schwein.) Hyl.)
Hordeum jubatum L. v. *caespitosum* (Scribn.) Hult.
Elymus glaucus Buckl.
Helictotrichon Hookeri (Scribn.) Henr.
Oryzopsis hymenoides (R. & S.) Ricker
O. micrantha (T. & R.) Thurb.
Stipa spartea Trin. v. *curtiseta* Hitchc.
S. comata T. & R.
S. viridula Trin.
S. Richardsonii Link.
Aristida longiseta Steud.
Muhlenbergia cuspidata (Nutt.) Rydb.
Calamovilfa longifolia (Hook.) Scribn.
Calamagrostis montanensis Scribn.
Bouteloua gracilis (HBK.) Lag.
Schedonnardus paniculatus (Nutt.) Trel.
Spartina gracilis Trin.
Buchloe dactyloides (Nutt.) Engelm.
Andropogon Hallii Hack.
Eleocharis Engelmannii Steud. v. *monticola* (Fern.) Svenson
Scirpus paludosus Nels.
S. pallidus (Britt.) Fern.
Carex Douglasii Boott.
C. stenophylla Wahlenb. v. *enervis* (Mey.) Kükenth.
C. praegracilis Boott.
C. Hookeriana Dew.
C. festivella Mack.
C. xerantica Bailey
C. saximontana Mack.
C. filifolia Nutt.
C. obtusata Lilj.
C. Rossii Boott.
C. Torreyi Tuckerm.
C. Hallii Olney
C. laeviconica Dew.
Tradescantia occidentalis (Britt.) Smyth
Allium textile Nels. & Mack. (Thunder Bay)
A. stellatum Fraser
Lilium umbellatum Pursh
Disporum trachycarpum (Wats.) B. & H.
Zygadenus elegans Pursh

Dicotyledons

Populus Sargentii Dode
Salix lutea Nutt.
S. rigida Muhl.
Comandra pallida A. DC.
Eriogonum flavum Nutt.
Rumex venosus Pursh
Polygonum interior Brenkle
Salicornia rubra Nels.
Eurotia lanata (Pursh) Moq.
Sueda depressa (Pursh) Wats.
Corispermum nitidum Kit.
C. simplissimum Lunnell
C. orientale Lam. v. *emarginatum* (Rydb.) Mack.
Cycloloma atriplicifolium (Spreng.) Coult.
Atriplex Nuttallii Wats.
A. argentea Nutt.
Monolepis Nuttaliana (R. & S.) Greene
Chenopodium leptophyllum Nutt.
C. Fremontii Wats.
C. Berlandieri Moq.
Amaranthus albus L.
A. angustifolium Lam.; Tell. (syn.: *A. graecizans* L.)
Oxybaphus nyctaginea (Michx.) Sw. (syn.: *Mirabillis nyctaginea* (Michx.) MacM.)
O. hirsutus (Pursh) Sw. (syn.: *M. hirsitis* (Michx.) MacM.)
Melandrium Drummondi (Wats.) Porsild
Ranunculus Cymbalaria Pursh v. *saximontana* Fern.
R. rhomboideus Goldie
Cleome serrulata Pursh
Draba reptans (Lam.) Fern. v. *micrantha* (Nutt.) Fern.
D. nemorosa L. v. *lejocarpa* Lindbl.
Lesquerella ludoviciana (Nutt.) Wats.
Lepidium ramosissimum Nels.
Thellungiella salsuginea (Pall.) O. E. Schultz (syn.: *Arabidopsis glauca* (Nutt.) Rydb.)
Erysimum asperum (Nutt.) DC.
Arabis Holboellii Hornem. v. *Collinsii* (Fern.) Rollins
Heuchera Richardsonii R. Br. (Quetico)
Rosa Woodsii Lindl.
Geum triflorum Pursh
Potentilla paradoxa Nutt.
P. rivalis Nutt.
P. millegrana Engelm.
P. pentandra Engelm.
Potentilla flabelliformis Lehm.

TABLE IV (*Continued*)

P. gracilis Dougl. (incl. v. *pulcherrima* (Lehm.) Fern.) (Thunder Bay)
P. concinna Richardson
P. effusa Dougl.
P. Hippiana Lehm. (Thunder Bay)
P. plattensis Nutt.
P. pensylvanica L. (incl. v. *arida* Boivin) v. *bipinnatifida* (Dougl.) T. & G.
Amelanchier alnifolia Nutt.
Thermopsis rhombifolia (Nutt.) Rich.
Lotus americanus (Nutt.) Bisch.
Psoralea argophylla Pursh
P. esculenta Pursh
Amorpha nana Nutt.
A. canescens Pursh
A. fruticosa L. v. *angustifolia* Pursh
Petalostemum candidum (Willd.) Michx.
P. occidentale (Gray) Fern.
P. purpureum (Vent.) Rydb.
P. villosum Nutt.
Oxytropis sericea Nutt. v. *spicata* (Hook.) Barneby
O. gracilis (Nels.) K. Schum.
O. Lambertii Pursh
Astragalus triphyllus Pursh
A. carnosus Pursh
A. lotiflorus Hook.
A. flexuosus Dougl.
A. bisulcatus (Hook.) Gray
A. missouriensis Nutt.
A. pectinatus Dougl.
A. striatus Nutt. (Thunder Bay)
A. goniatus Nutt.
Glycyrrhiza lepidota (Nutt.) Pursh
Hedysarum alpinum L. v. *philoscia* (A. Nels.) Rollins
Vicia americana Muhl. v. *truncata* (Nutt.) Brewer
v. *angustifolia* Nees
Linum rigidum Pursh
Euphorbia Geyeri Engelm.
Sphaeralcea coccinea (Pursh) Rydb.
Viola pedatifida G. Don.
V. Nuttalii Pursh
V. rugulosa Greene
Menzelia decapetala (Pursh) Urban & Gilg.
Opuntia fragilis (Nutt.) Haw. (W. Ontario)
O. polyacantha Haw.
Mamillaria vivipara (Nutt.) Haw.
Shepherdia argentea Nutt.
Gaura coccinea Pursh
Epilobium paniculatum Nutt.
Oenothera serrulata Nutt.
Oe. Nuttallii Sweet
Cymopterus acaulis (Pursh) Raf.
Lomatium foeniculaceum (Nutt.) C. & R.
L. macrocarpum (H. & A.) C. & R. (syn.: *Cogswellia macrocarpa* (Nutt.) Jones)
Musineon divaricatum (Pursh) Nutt.
Androsace occidentalis Pursh
A. septentrionalis L. v. *puberulenta* (Rydb.) Knuth.
Gentiana procera Holm.
G. Macounii Holm. (James Bay).
Apocynum sibiricum Jacq. v. *salignum* (Greene) Fern.
Asclepias viridiflora Raf.
A. speciosa Torr.
A. ovalifolia Dcne.
Cuscuta megalocarpa Rydb.
Heliotropium curassavicum L. v. *obovatum* A. DC.
Lithospermum canescens (Michx.) Lehm. (Thunder Bay)
L. incisum Lehm.
Lappula Redowskii (Hornem.) Greene v. *occidentalis* (Wats.) Rydb.
Onosmodium occidentale Mack.
Allocarya californica (F. & M.) Greene
Verbena bracteata Lag. & Rodr.
Agastache foeniculum (Pursh) Ktze. (Quetico, Thunder Bay)
Dracocephalum formosius (Lunell) Rydb.
D. Nuttallii Britt.
Salvia reflexa Hornem.
Lycopus asper Greene
Solanum rostratum Dunal
S. triflorum Nutt.
Penstemon nitidus Dougl.
P. albidus Nutt.
P. gracilis Nutt.
Castilleja miniata Dougl.
C. sessiliflora Pursh
Orthocarpus luteus Nutt. (N. Minn., Thunder Bay)
Orobanche fasciculata Nutt.
O. ludoviciana Nutt.
Plantago Purshii R. & S.
P. elongata Pursh
Liatris punctata Hook.
Grindelia squarrosa (Pursh) Dunal
Gutierrezia diversifolia Greene
G. sarothrae (Pursh) Britt. & Rusby
Chrysopsis villosa (Pursh) Nutt. (incl. *C. Bakerii* Greene and *C. Ballardi* Rydb.)
C. hispida (Hook.) DC.
Solidago missouriensis Nutt. (incl. vars.) (Thunder Bay)
S. nemoralis Ait. v. *decemflora* (DC.) Fern. (Thunder Bay)
S. mollis Bartl.
S. gilvocanescens (Rydb.) Smyth
S. canescens Rydb. (syn.: *S. rigida* L. v. *humilis* Porter)
Haplopappus spinulosum (Pursh) DC.
Aster umbellatus Mill. v. *pubens* Gray (James Bay)
A. paucifolius Nutt.
A. ericoides L. s. lat.
A. commutatus (T. & G.) Gray
Townsendia exscapa (Rich.) Porter
Erigeron asper Nutt.
Iva axillaris Pursh
I. xanthifolia Nutt.
Ambrosia coronopifolia T. & G. (Thunder Bay)
Rudbeckia serotina Nutt. (Quetico)
Echinacea angustifolia DC.
Ratibida columnifera (Nutt.) Woot. & Standl.
Helianthus annuus L.

TABLE IV (*Concluded*)

H. petiolaris Nutt.
H. laetiflorus Pers. v. *rigidus* (Cass.) Fern. v. *subrhomboideus* (Rydb.) Fern.
H. Maximiliani Schrad. (Quetico, Thunder Bay)
Coreopsis tinctoria Nutt.
Madia glomerata Hook.
Helenium autumnale L. v. *montanum* (Nutt.) Fern.
Gaillardia aristata Pursh
Artemisia cana Pursh
A. glauca Pall. (incl. *A. Dracunculus* L. ssp. *glauca* (Pall.) Hall. & Clem. and *A. dracunculoides* Pursh)
A. caudata Michx. v. *Richardsiana* (Bess.) Boivin (Quetico, Thunder Bay)
A. longifolia Nutt.
A. ludoviciana Nutt. v. *gnaphalodes* (Nutt.) T. & G. (Quetico)
v. *pabularis* (Nels.) Fern. (Quetico, Thunder Bay)
Arnica fulgens Pursh
Senecio eremophilus Richards.
S. integerrimus Nutt.
S. canus Hook.
S. tridenticulatus Rydb. (syn.: *S. densus* Greene)
Cirsium Flodmanii (Rydb.) Arth.
Lactuca Pulchella (Pursh) DC. (Quetico, Thunder Bay, James Bay)
Lygodesmia juncea (Pursh) G. Don
Agoseris cuspidata (Pursh) Raf.
A. agrestis Osterh.
A. glauca (Nutt.) Greene v. *dasycephala* Jepson
Crepis runcinata (James) T. & G. (incl. v. *glauca* (Nutt.) Babcock & Stebbins)

still open to migration. The turtles, at least the painted one, must have followed the waterways, and their densest concentration is now the Winnipeg River drainage area in the southeastern corner of the province. They are found as far north as the 51° Lat. north, but above that summers are now too short for their eggs to hatch, and even further south some summers prove fatal to the eggs (5). The turtles now lead a somewhat precarious existence on their northern limit in Manitoba.

Cacti are found in southwestern Manitoba in several localities and there always in dry, sandy hills and plains (60, 88). The most common is *Opuntia polyacantha*, but *Mamillaria vivipara* is locally abundant. Both species exist in similar habitats in Saskatchewan and North Dakota, and the latter species has one locality in Minnesota in the vicinity of the old Lake Traverse outlet (Bigstone Co., near Minnesota River; G. Ownbey, personal communication). The third species, *Opuntia fragilis*, does not occur in Minnesota. All three species are found in the Spruce Woods Forest Reserve, the old "Assiniboine Delta," where they grow in a habitat of living sand dunes that can only be described as a "desert". In the outskirts of the true desert habitat, *Mamillaria* grows on stabilized dunes, over which white spruce have encroached. One has thus the unique opportunity of stepping on sharp cactus spines when approaching a sturdy spruce tree.

Opuntia polyacantha grows also farther east than this, in the Red River Valley, not far from Morden, on alkaline prairie (60, 88). The most easterly locality in Manitoba for cactus is, however, the dry granite shores of Falcon Lake on the Ontario border in the Whiteshell Forest Reserve, where *Opuntia fragilis* thrives. This species reaches even into western Ontario, where Mr. Rodney Housha found it growing in moss on an island in Rainy Lake, 15 miles from International Falls (G. Ownbey, personal communication). This locality is within the former Lake Agassiz area. *Opuntia fragilis* is also found in several localities along the Minnesota River Valley, around the former southern lake outlet.

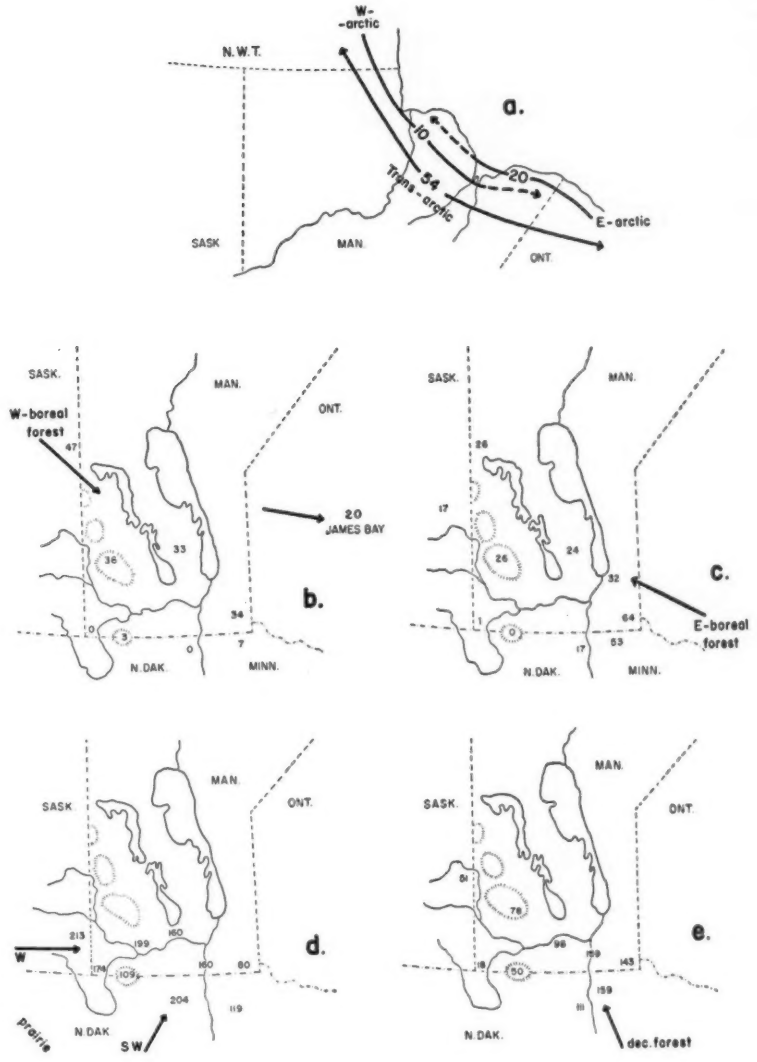


FIG. 4. Graphic representation of various "elements" in the flora of Manitoba. The figures represent numbers of taxa (compiled from direct observation and literature) in approximately the area where they are placed on the maps: (*a*) "arctic" (p. 575), (*b*) "west-boreal" (p. 579), (*c*) "east-boreal" (p. 578), (*d*) "western" (p. 569), and (*e*) "eastern" (p. 562) flora elements.

Many other prairie species followed the eastwards spillways. Unfortunately, western Ontario is, from a botanical point of view, one of the least known, and least accessible, areas of southern Canada, but some very fine collections have been made in the Thunder Bay district and in Quetico Park by Dr. T. M. C. Taylor (93, 94) and Mr. C. E. Garton (40). With their consent I have been able to draw on information from unpublished lists, and I have chosen the following species as examples of eastwards extensions of western species, which can have arrived during the hypsithermal to the vicinity of Lake Superior. All localities in which these species have been collected are truly "wild" and "uncontaminated" by man. (Quet. = Quetico Park, coll. Garton; Sibl. = Sibley Peninsula, Thunder Bay, coll. Taylor, Garton; Schr. = Schreiber area, Thunder Bay, coll. Taylor.)

Festuca saximontana (Quet., Schr.), *Andropogon Gerardi* (Quet.), *Carex foena* (Sibl., Quet.), *Conringia orientalis* (Sibl.), *Erysimum inconspicuum* (Sibl., Schr.), *Heuchera Richardsonii* (Quet.), *Potentilla pensylvanica* v. *glabrata* (Sibl., Quet.), *P. Hippiana* v. *Hippiana* (Sibl.), *P. gracilis* v. *pulcherrima* (Sibl.), *Astragalus striatus* (Sibl.), *Convolvulus spithamaeus* (Quet.), *Phacelia Franklinii* (Sibl.), *Lithospermum canescens* (Sibl.), *Orthocarpus luteus* (Sibl.), *Gerardia tenuifolia* v. *parviflora* (Quet.), *Agastache Foeniculum* (Quet.), *Solidago nemoralis* v. *decemflora* (Sibl.), *Artemisia ludoviciana* v. *pabularis* (Quet.), and *Artemisia caudata* v. *Richardsiana* (Sibl., Quet.).

More species than these, and more localities for some, are given by Taylor and Garton (personal communication), but many of such a nature that introduction by man cannot be excluded, e.g., localities in the cities of Port Arthur and Fort William, along highways and railways.

The north shore of Lake Superior is interesting not only for its disjunct prairie species but also for housing several "cordilleran" and "arctic" species (cf. Fernald (36)). All seem to appear in the immediate vicinity of and in the area where Lake Agassiz drained (31), and it is tempting to think that they all in some way are connected with the Lake Agassiz problem. It still remains to be investigated whether the true arctic species among those listed by Fernald (36) could possibly have come south as late as after the northwards outlet of Lake Agassiz opened, as some sort of an extension of a coastal arctic element from submerged James Bay to the vicinity of Lake Superior. The distance from, e.g., Lake Nipigon to the submerged James Bay was not very great, and to the Ogoki River system even shorter. Many of the arctic species are also found around Lake Nipigon, and all the true arctic species around Lake Superior are also found in James Bay (cf. Fernald (36); Dutilly, Lepage, and Duman (29, 30)). It must also be remembered that some arctic plants are more specific as to habitat than to temperature and day length (compare *Koenigia* studies by Löve and Sarkar (59); Dahl (22)).

The possibility for such a condition to develop hinges of course on the existence of an arctic flora in James Bay, having reached down already from further north. When the northern outlets opened, the Laurentian ice sheet had split in two lobes, the Keewatin and the Labrador residual sheets (31). These remained inland longer than on the coast, and may have had periods

of both expansion and waning (cf. Lawrence (52)), but not reaching the coast. After the ice load disappeared, the coast around the submerged Hudson Bay began to rise (38), and at least then, if not before, ample opportunity existed for a southwards migration over barren beaches for an arctic flora which had survived in unglaciated areas farther north (47, 48, 56, 58).

Manitoba has ca. 85 species in common with the Arctic Archipelago (cf. Ritchie (80); Porsild (75); Scoggan (88)), of which most, ca. 55 species, are found from west to east in the American Arctic (Fig. 4*a*). Smaller numbers belong to species with a more distinctly western (10 species) or eastern (20 species) distribution area (cf. Table V). In addition, there are some endemics: *Carex Dutillyi* and *Oxytropis Bellii*, both mainly on the western side of Hudson Bay (75, 88), and *Carex oligosperma* v. *churchilliana* and *Melandrium Gillettii*, known only from Churchill (80).

Of the 85 species, 15 have not been found in James Bay (29, 30, 75, 88). It is not surprising that four of these are "west arctic" species, which have not

TABLE V

List of taxa belonging to a "high-arctic element," reaching Manitoba from the north, northwest, or northeast, mostly along the coast of Hudson Bay, but penetrating somewhat inland

(*a*) Taxa in Manitoba which have a general distribution in the American–Canadian Arctic

Monocotyledons

Puccinellia phryganodes (Trin.) Scribn. & Merr.
Festuca rubra L. v. *arenaria* (Osb.) Fr. (= *F. Richardsonii* R. Br.)
Arctophila fulva (Trin.) Rupr.
Dupontia psilosantha Rupr.
Hierochloë alpina (Sw.) R. & S.
Eriophorum Scheuchzeri Hoppe
E. russeolum Fr.
E. callitrix Cham.
Kobresia simpliuscula (Wahlenb.) Mack.
Carex maritima Gunn.
C. glareosa Wahlenb. v. *amphigena* Fern.
C. amblyorhyncha Krecz.
C. rupestris All.
C. glacialis Mack.
C. bicolor All.
C. atrofusca Schk.
C. rariflora (Wahlenb.) Sm.
C. subspatacea Wormskj.
C. microglochin Wahlenb.
Juncus biglumis L.
J. albescens (Lge.) Fern.
Luzula confusa Lindebl.

Dicotyledons

Salix reticulata L.
S. arctophila Cockll.
Bistorta vivipara (L.) Gray (syn.: *Polygonum viviparum* L.)
Sagina nodosa (L.) Fenzl.
Arenaria peploides L. v. *diffusa* Hornem. (syn.: *Honkenya diffusa* (Hornem.) Löve)
A. rubella (Wahl.) Sm. (syn.: *Minuartia rubella* (Wahl.) Hiern.)
A. uliginosa Schleich (syn.: *Minuartia stricta* (Sw.) Hiern.)
Stellaria monantha Hult.
S. ciliatosepala Trautv.
S. humifusa Rottb.
Melandrium apetalum (L.) Fenzl. ssp. *arcticum* (Fr.) Hult.
M. affine (J. Vahl) Hartm.
Ranunculus trichophyllus Chaix. v. *eradicatum* (Laest.) W. B. Drew (syn.: *Batrachium trichophyllum* Schultz var...)
R. Pallasii Schlecht.
R. pedatifidus Sm. v. *leiocarpus* (Trautv.) Fern.
Draba alpina L.
D. nivalis Liljebl.
D. glabella Pursh
Lesquerella arctica (Wormskjold) Wats.
Cochlearia officinalis L. s. lat. (*C. groenlandica* L. ?)
Saxifraga Hirculus L.
S. aizoides L.
S. oppositifolia L.
Potentilla nivea L. s. lat.
P. pulchella Pursh
Hippuris tetraphylla L. f.
Ledum decumbens (Ait.) Lodd
Lomatogonium rotatum (L.) Fries
Mertensia maritima (L.) S. F. Gray
Pedicularis lapponica L.
P. labradorica Wirsing
Matricaria ambigua (Ledeb.) Kryl.

TABLE V (*Concluded*)

(*b*) Taxa in Manitoba which have a general distribution in the western part of the American–Canadian Arctic

Monocotyledons

Puccinellia lucida Fern. & Weath.
Calamagrostis deschampsioides Trin. (James Bay)
Agropyron latiglume (Scribn. & Sm.) Rydb.
Luzula Wahlenbergii Rupr.

Dicotyledons

Salix alaxensis (Anderss.) Cov.
S. niphoclada Rydb.
Descurainia sophioides (Fisch.) Schulz
Arabis arenicola (Rich.) Gel. v. *pubescens* (Wats.) Gel.
Pedicularis sudetica Willd. (James Bay)
Artemisia Tilesii Ledeb. v. *elatior* T. & G. (James Bay)

(*c*) Taxa in Manitoba which have a general distribution in the eastern part of the Canadian Arctic

Monocotyledons

Puccinellia vaginata (Lge.) Fern. v. *paradoxa* Th. Sør.
P. Langeana (Berl.) Th. Sør.
Carex capitata L.
C. rufina Drej.
C. norvegica Retz.
C. Bigelowii Torr.
C. paleacea Wahlenb.
C. saxitilis L. (v. *rhomalea* Fern.) (Not in James Bay!)
Juncus arcticus Willd.

Dicotyledons

Salix herbacea L.
S. cordifolia Pursh ssp. *callicarpea* (Trautv.) Löve
S. calcicola Fern.
Sagina caespitosa (J. Vahl.) Lge. (Not in James Bay!)
Cerastium alpinum L.
Saxifraga caespitosa s. lat.
Oxytropis terrae-novae Fern. (Not in James Bay!)
Diapensia lapponica L. (Not in James Bay!)
Euphrasia borealis (Towns.) Wettst. (syn.: *E. arctica* Lge.)
Bartsia alpina L.
Pedicularis flammea L.

yet reached that far east (*Luzula Wahlenbergii*, *Salix alaxensis*, *S. niphoclada*, and *Descurainia sophioides*), but five have an otherwise wide area in the far north (*Stellaria monantha*, *S. ciliatosepala*, *Arenaria uliginosa*, *Melandrium affine*, and *Lesquerella arctica*). Most remarkable is the occurrence in Manitoba of five "east-arctic" species, which are not (so far?) found in James Bay (*Carex rhomalea*, *Salix herbacea*, *Sagina caespitosa*, *Oxytropis terraenovae*, and *Diapensia lapponica*). This makes one wonder whether most of the "arctic element" has not come to Manitoba along the west side of Hudson Bay, even the species from the American east-arctic?

Most of the high-arctic element is coastal, but some is found on the barren land of northwestern Manitoba on the border of the Northwest Territories, and a few reach as far south and inland in this province as Gillam on the Nelson River and points along Hayes River (*Eriophorum Scheuchzeri*, *Carex microglochin*, *Polygonum viviparum*, *Sagina nodosa*, *Arenaria uliginosa*, *Draba glabella*, *Saxifraga Hirculus*, and *Artemisia Tilesii* v. *elatior*), which may be an indication of the southernmost extension in Manitoba of the submerged Hudson Bay.

In Manitoba the last piece of land to be released from the grip of the continental ice may thus not have been the coastal strip of "tundra," but rather the northwestern part of the province from Nelson River up towards the barren land in the Northwest Territories (Fig. 2), roughly corresponding to zone B 27 of Halliday (42). This may have occurred as late as towards the end of the hypsithermal period, and may be the cause of the poorly developed nature of the area, perhaps most properly described by Hustich's (49) term "taiga." Zone B 27 has a clear western affinity, which even reaches through zone B 5 (cf. Halliday (42); = Hudson Bay Lowland, cf. Ritchie (82)) as far as to James Bay, whereas the zones B 21 and B 22*a*, south of them, exhibit more of an eastern influence which, however, does not penetrate so solidly westwards into Saskatchewan.

TABLE VI

List of taxa belonging to an "east-boreal element" reaching from east into Manitoba, occasionally as far west as to N. Saskatchewan

Monocotyledons

Potamogeton epihydrus Raf. v. *Nuttallii* (C. & S.) Fern. (bicentr.)
Najas flexilis (Willd.) Rostk. & Schmidt (bicentr.)
Sagittaria rigida Pursh
Eleocharis Smallii Britt.
E. elliptica Kunth.
Rhynchospora capillacea Torr. (Sask.)
Carex argyrantha Tuckerm.
C. gracillima Schwein.
C. castanea Wahl.
C. pseudo-cyperus L. (Sask.)
Symplocarpus foetidus (L.) Nutt.
Juncus brevicaudatus (Engelm.) Fern. (Sask., Alta.)
Clintonia borealis (Ait.) Raf.
Iris versicolor L.
Cypripedium reginae Walt.
Arethusa bulbosa L.
Platanthera Hookeri (Torr.) Lindl.
Spiranthes gracilis (Bigel.) Beck. (Sask.)
Malaxis brachypoda (Gray) Fern. (Sask.)
M. unifolia Michx.
Calopogon pulchella (Salisb.) R. Br.

Dicotyledons

Populus balsamifera L. v. *subcordata* Hyl.
Salix lucida Muhl. (Sask.)
S. humilis Marsh (Sask.)
S. vestita Pursh (Bicentr.)
Myrica Gale L. s. lat.
Alnus rugosa (DuRoi) Spreng. v. *americana* (Regel) Fern. (Sask.)
Arceuthobium pusillum Peck.
Polygonum boreale (Lge.) Small (bicentr.)
Atriplex glabriuscula Edmonston
Nymphaea odorata Ait.
Nuphar microphyllum (Pers.) Fern.
Thalictrum confine Fern.
Ranunculus septentrionalis Poir.
Draba minganensis (Vict.) Fern.
Draba incana L. v. *confusa* (Enrh.) Liljebl.
Parnassia glauca Raf.
Ribes hirtellum Michx. (et vars.)
Potentilla pensylvanica L. v. *pectinata* (Raf.) Lepage (syn.: *P. litoralis* Rydb.) (Alta.; bicentric?)
Sorbus decora (Sarg.) Lehm.
Oxytropis Johannensis Fern.
Lathyrus maritimus (L.) Bigel. v. *glaber* (Ser.) Eames (bicentr.)
Hudsonia tomentosa Nutt. (Sask.)
Lechea intermedia Leggett (Sask.)
Oenothera perennis L.
Oe. parviflora L. (Sask.)
Aralia hispida Vent. (Sask.)
Andromeda glaucophylla Link.
Gaultheria procumbens L.
Epigaea repens L. v. *glabrifolia* Fern.
Vaccinium angustifolium Ait. (et vars.)
Gentiana linearis Fröl.
Lonicera oblongifolia (Goldie) Hook. (Sask.)
L. villosa (Michx.) R. & S. v. *Solonis* (Eat.) Fern.
Diervilla Lonicera Mill. (Sask.)
Campanula uliginosa Rydb.
Solidago hispida Muhl. v. *lanata* (Hook.) Fern. (Sask.)
S. Purshii Porter
Aster Johannensis Fern.
A. simplex Willd. (Sask.)
Antennaria canadensis Greene (Sask.)
A. petaloidea Fern.
A. neodioica Greene

As mentioned before, it is extremely difficult to establish taxonomically from where the trees came, but if we study the accompanying undervegetation it seems that more "western boreal" species invaded along the northern edge of the conifer forest and that more "eastern boreal" species came from the Great Lakes area and followed around the north ends of the prairie lakes. Unfortunately, the undervegetation of the coniferous forest is a very homogeneous and rather scanty element, and most of its species range over wide areas. We have, however, distinguished 65 species in the flora of Manitoba, which we call the "east boreal" element (cf. Table VI; Fig. 4*c*), having most of its species concentrated east of Manitoba. especially north of the Great Lakes. Seventeen of these 65 reach Saskatchewan, but only four of them, *Juncus brevicaudatus*, *Hudsonia tomentosa*, *Lechea intermedia*, and *Aralia hispida*, are found as far west as Athabasca Lake (according to Fraser and Russell (39); Raup (79)), and only one, *Juncus brevicaudatus* (according to

TABLE VII

List of species belonging to a "west-boreal element" reaching from (Alaska and) northwestern Canada from northwest into Manitoba, occasionally as far southeast as to James Bay

Monocotyledons

Elymus innovatus Beal. (James Bay)
Calamagrostis purpurascens R. Br.
Eriophorum gracile Koch (v. *caurianum* Fern?) (James Bay)
Carex loliacea L. (James Bay)
C. Raymondii Calder
C. rotundata Wahl.
Cypripedium passerinum Richards (James Bay)

Dicotyledons

Salix pseudomonticola Ball. (James Bay)
S. athabascensis Raup
S. Scouleriana Barrat
S. arbusculoides Anderss.
Myrica Gale L. s. lat.
Betula papyrifera Marsh v. *humilis* (Regel) Fern. & Raup (syn.: v. *neoalaskana* (Sarg.) Raup)
Arceuthobium americanum Nutt.
Claytonia Bostockii Porsild
Stellaria longifolia Muhl. v. *atrata* Moore (James Bay)
Silene Menziesii Hook.
Aquilegia brevistyla Hook. (James Bay)
Caltha natans Pall.
Thalictrum sparsiflorum Turcz. v. *Richardsonii* (Gray) Boivin
Thalictrum venulosum Trel. (James Bay)
Ranunculus Gmelinii DC. v. *limosus* (Nutt.) Hara
Ribes oxyacanthoides L. (James Bay)
Geum macrophyllum Willd. v. *perincisum* (Rydb.) Raup (James Bay)
Potentilla pensylvanica L. v. *glabrata* (Hook.) Wats. (syn.: *P. glabrella* Rydb.) (James Bay)
Oxytropis deflexa (Pall.) DC. v. *sericea* T. & G.
O. campestris (L.) DC. v. *varians* (Rydb.) Barneby
O. viscida Nutt. v. *hudsonica* (Greene) Barneby (James Bay) (sometimes regarded as a synonym of preceding sp.)
Astragalus yukonis M. E. Jones
A. americanus (Hook.) M. E. Jones
Hedysarum Mackenzii Richardson
Impatiens Noli-tangere L.
Cicuta Mackenzieana Raup (James Bay)
Chimaphila umbellata (L.) Bart. v. *occidentalis* (Rydb.) Blake (James Bay)
Pyrola grandiflora Radius v. *canadensis* (Andres) Porsild
Mertensia paniculata (Ait.) G. Don (James Bay)
Castilleja Raupii Pennell (James Bay)
Pedicularis parviflora Sm.
Adoxa Moschatellina L.
Solidago decumbens Greene v. *oreophila* (Rydb.) Fern.
Aster modestus Lindl. (James Bay)
Erigeron lonchophyllus Hook. (James Bay)
Antennaria campestris Rydb. v. *athabascensis* Boivin
A. rosea (D. C. Eat.) Greene (James Bay)
Arnica Chamissonis Less. ssp. *foliosa* (Nutt.) Maguire (James Bay)
A. lonchophylla Greene
Cirsium Drummondi T. & G.

Scoggan (88)), reaches northern Alberta. *Juncus* and *Aralia* seem to have continuous areas all the way, but doubt as to the continuity of *Hudsonia* and *Lechea* seem warranted, as no localities have been established for them between southeastern Manitoba, east of Lake Agassiz basin, and Lake Athabasca, Saskatchewan.

On the other hand, out of the 48 species in Manitoba, which we consider "west boreal" (cf. Table VII; Fig. 4*b*), not less than ca. 21 have been reported from James Bay (29) and as many as 13 even on the east side of that bay (30), indicating a rather intense migration from the west: *Eriophorum gracile* v. *caurianum*, *Cypripedium passerinum*, *Salix pseudomonticola*, *Stellaria atrata*, *Aquilegia brevistyla*, *Ribes oxyacanthoides*, *Oxytropis viscida* v. *hudsonica* (= *varians* in Scoggan (88)), *Cicuta Mackenzieana*, *Mertensia paniculata*, *Castilleja Raupii*, *Pedicularis parviflora*, *Antennaria rosea*, and *Arnica Chamissonis* v. *angustifolia*.

However, the region between the north ends of the prairie lakes and the Hudson Bay Lowlands is not yet well known, and only some limited portions have been thoroughly studied, as e.g. the MacBride Lake – Tod Lakes areas by Ritchie (81). Other regions where botanical collections and ecological observations have been made are the Reindeer – Nueltin Lakes area in northwestern Manitoba (3), the Hayes River route (87), and the area around Norway House and Grands Rapids (86). More work is needed here to establish with some degree of certainty from where the forest actually invaded this part of the province, but there is no doubt that Manitoba can be considered as the meeting place and melting pot of eastern and western boreal forest in Canada, and that the most intense mixing has taken place as late as from the hypsithermal to the present time, i.e., during the last 4000 years.

The Period 2000 B.P. – The Present

By 2000 B.P. certainly all residual ice had vanished from Manitoba, and it does not seem that this area was noticeably affected by any glaciation in connection with the so-called "Little Ice-Age" (53). The hypsithermal had reached its peak sometime around 6000 to 4000 B.P. but from then on a slow but steady decrease in mean temperature took place. Around 2000 B.P. the deterioration had visibly effected a shift in vegetation belts, and the hypsithermal period is definitely over (28). In eastern United States oak and beech forests were being invaded by hemlock, spruce, and chestnut (26), and in Canada this time meant more than anything else an expansion of spruce, which still is in progress (Fig. 2).

In Manitoba around 2000 B.P. the Indians at the Lockport site (see p. 569), who had previously only hunted bison, began to leave bones of woodland animals (elk, deer, etc.) on their refuse heaps, and from around 1000 B.P. also moose bones (MacNeish, personal communication). This indicates a gradual changing of their surrounding habitat from a prairie to a woodland to an approaching forest (Fig. 3).

The area of true prairie is now gradually diminishing, and the aspen zone is growing out over it, perhaps even better expressed as "under it," as the invasion seems to be rather by root sprouting than by seeding (68). Were it not for the raging prairie fires (in early days often set by man) the encroachment of forest upon prairie would have been still more visible today (7, 34, 68, 69, 71). The extent of black soil under much of the present aspen belt tells the story of the recent expansion of aspen over grassland (68, 71), as do the patches of "prairie" left inside the present forest belt (e.g., in Riding Mountains, Rowe (84); in Sandilands Forest Reserve, present author). Also in Minnesota the same expansion of hardwoods over prairie and black soil, more or less checked by fire, is clearly visible (13; Buell, personal communication).

In the wake of the south-moving aspen belt, and mixing with it, followed the spruce forest, now actively expanding in all directions, southwards as well as north-, east-, and west-wards. Along its southern border, it mixed with the hardwoods, and the typical "mixed woods" of Halliday (42), or "southern boreal forest" of Rowe (84), may have assumed the aspect it has in Manitoba today: an intricate quilt work of northern hardwood and coniferous trees, each occupying habitats most suited for their particular needs, and accompanied by an undervegetation typical for both. Further south, in the Spruce Woods Forest Reserve, the spruce is encroaching on the sand dunes (7). Still further south, in Minnesota, Buell and Gordon (14) have noticed the same trend in Itasca Park, where spruce–fir forest is currently invading the maple–basswood stands (cf. also Buell and Bormann (12)), and in Wisconsin Wilson (101) found a similar development from hardwood into a mixed second growth pine–hardwood forest over old prairie. In the northwest, around Athabasca and Great Slave Lake, Raup (78) has observed a "recent" spruce invasion into the open pine–savana, which earlier had replaced the original late-glacial spruce parkland.

During the last couple of centuries the White Man has been on the scene to watch the changing nature and to help promote the changes himself. Hudson Bay was discovered in the early 1600's and then the strand line was further inland than today. La Verendrye and his sons (16) had difficulties finding pitch to repair their canoes when, towards the end of the 1700's, they reached the lower part of Roseau River in southeastern Manitoba, where today the spruce and pine grow within a stone's throw of the river. But they were able to take their canoes from Portage la Prairie over to Lake Manitoba without too much difficulty, through marshes where we now have wheatfields. The first white settlement was founded in 1812 by Scotchmen, Fort Garry in the Red River Valley (now Winnipeg); its "Main Street" was an old "buffalo trail."

A both interesting and valuable source of information on the changing landscape can be gained from reports by factors and clerks in the trading companies as well as memories written down by the early settlers. Cowie (21), a clerk in Hudson's Bay Company, travelled in Manitoba and Saskatchewan between 1867 and 1874, and in the Qu'appelle Valley he observed "among the prevailing poplar a stray oak and a chance spruce," the spruce probably being the last remnants of the riverine spruces so far south. French

settlers on the east side of the Red River have given vivid descriptions (50, 90) of how they changed their land from open prairie and mosquito-infested swamps and marshes, where the only means of transportation was the Red River cart with huge wheels of oak and bisonhide-tongues, to a rich farmland by draining and plowing. They also describe how the elm–tamarack swamps in "Cypress Mountain" (now Sandilands Forest Reserve) were ruined by lumbering, draining, and encroachment of black and white spruce.

When looking out over the level plains and gently rolling hills of modern Manitoba south of the prairie lakes, where wheat is today's "prairie grass," it is hard to imagine that a mere 7000 years ago this was the bottom of a deep ice-dammed lake.

Summary

1. All vegetation of Manitoba was obliterated during the Wisconsin Ice Age.

2. It is postulated that the glacier reached the grassland on the western plains, having overtaken the forests, but that a coniferous forest reserve with some northern hardwoods existed at the foot of the Rocky Mountains in the west, and another reserve south of the ice in eastern and central United States, separated from the grassland by a belt of deciduous forest.

3. 11,000–9000 B.P. A "northern" (*Festuca–Poa–Agropyron* s. lat.) grassland follows the ice withdrawal, and a "marsh–grassland" develops on the bottom of the drained Lake Agassiz I during the Two Creeks interval. A riverine spruce parkland of western origin develops along drainage channels to the Lake Agassiz basin. This flora persists throughout the advance and early retreat of the Valders ice substage.

4. 9000–6000 B.P. A deciduous (*Ulmus–Fraxinus–Populus*) forest develops around the shores of Lake Agassiz II and its drainage channels at the same time as a pine–oak (*Pinus Banksiana – Quercus macrocarpa*) savanna spreads over drier upland. The origin of the pine forest is discussed, and reasons given that it may have both western and eastern distribution centra.

5. 6000–2000 B.P. During the hypsithermal period the southern deciduous forest reaches its northern and westernmost limits, and as Lake Agassiz II drains, first east- then north-wards, a western and southwestern prairie flora (*Stipa–Andropogon–Bouteloua*–leguminose–composite flora) invades the lake bottom and drainage channels from west and south. Along the shores of Hudson Bay an arctic flora migrates south, and spruce forests spread over the precambrian shield. The last area to be deglaciated (3000–4000 years ago?) seems to have been the "taiga" belt of northwestern Manitoba. The origin of the spruce forest in northern Manitoba is discussed. It seems to have come from both west and east.

6. 2000–present. The period is characterized by a spruce expansion in all directions. The southwards migration of spruce forest penetrates into the aspen belt and a mixed forest results. The aspen zone in turn invades the prairie south of it, and prairie land diminishes. During the last centuries Man helps transform southern Manitoba's prairies and marshes into a rich farmland.

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CYTOTAXONOMIC STUDIES IN THE GENUS ANEMONE[1]

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Abstract

Chromosome morphology has been described in 20 species of *Anemone* s. lat. (*Anemone* s. str. 15 species, *Pulsatilla* 3, *Hepatica* 2). Two series, one based on 8 chromosomes and the other on 7, are present, in each of which two patterns can be recognized when an orderly arrangement of the chromosomes has been made. In the 8-chromosome series, the distinctions are based on differences in the arm ratio of chromosome IV, namely ratios of about 1:2 versus 1:1.4–1.8. Species in the first group include *A. palmata*, $2n = 16$; *A. parviflora*, $2n = 16$; *A. caroliniana*, $2n = 16$; *A. silvestris*, $2n = 16$; *A. virginiana–riparia–cylindrica* complex, (all $2n = 16$); *A. quinquefolia*, $2n = 32$; and species in the second group are *A. pavonina*, $2n = 16$; *A. coronaria*, $2n = 16$; *A. hupehensis*, $2n = 16$; *A. rivularis*, $2n = 16$; *A. rupicola*, $2n = 16$; *Pulsatilla pratensis*, $2n = 16$; *P. nuttalliana* ssp., $2n = 16$; and *P. occidentalis*, $2n = 16$. *A. multifida*, $2n = 32$, has one chromosome set in each of the above groups. The 7-chromosome series falls into two groups on the basis of a combination of characters. Group one: long arms of chromosome I shorter than those of the longest chromosomes, long arms of chromosome II longer than those of the third pair, and arm ratios in chromosome II of 1.4–1.6. Group two: long arms of chromosome I longer than those of all others, long arms of chromosome II shorter than any of the others, arm ratios in chromosome II $< 1:1.4$. Species in the first group are *Anemone canadensis*, $2n = 14$; *A. richardsonii*, $2n = 14$; and *A. fasciculata*, $2n = 14$; and in the second group, *Hepatica americana*, $2n = 14$; and *H. acutiloba*, $2n = 14$. *Anemone multifida* and *A. quinquefolia* are recognized as allotetraploids. The cytological results are discussed in relation to current classification.

Introduction

A comprehensive study of the genus *Anemone* s. lat. was begun in 1955 as part of the program of the Botanical Garden, Department of Botany, University of Toronto, to obtain a better understanding of the individual species, their interrelationships, and the various categories into which they have been grouped. Methods include the examination of chromosome complements in root tips, hybridization experiments, and analysis of chromosome pairing in the resulting hybrids, supported by usual taxonomic procedures. This paper describes the results of root-tip study of species of *Anemone* s. lat., particularly of the categories established by some authors as *Anemone* s. str., *Pulsatilla*, and *Hepatica*, of the subtribe Anemoninae. It also discusses these results in relation to current taxonomic classification.

Taxonomic Survey and Earlier Cytological Work

Classification in the genus *Anemone* L. has long been a challenge to taxonomists. In 1891, Prantl (28) recognized two subgenera, subgenus Euanemone Prantl with sections Anemonanthea DC., Eriocephalus Hook. and Thoms., Homalocarpus DC., Hepatica Dill, Barneoudia Gay, and Knowltonia Salisb.; and subgenus Pulsatilla Tourn. with two sections, Preonanthus Ehrh. and Campanaria Endl.

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In 1892 Janczewski (15), stressing the importance of characteristics of the fruits as a basis of classification, recognized the following 10 sections: Hepatica Dill, Sylvia Gaud. (= Anemonanthea DC. in part), Omalocarpus DC., Anemonidium Spach, Rivularidium Jancz., Pulsatilloides DC., Pulsatilla Tourn., Eriocephalus Hook. and Thoms., Barneoudia Gay, and Knowltonia Salisb. The soundness of the definition of these categories is evident in the fact that subsequent revisions have been concerned chiefly with the relative status of these categories, or subdivisions within them, rather than with the species included.

Ulbrich's monograph of 1905/6 (30) restricted the interpretation of the genus *Anemone* by excluding *Pulsatilla*, *Knowltonia*, *Barneoudia*, and *Capethia* Britt. (= *Oreithales* Schlecht.). Within the genus *Anemone* s. str. two subgenera were recognized, Hepatica, and Euanemone with the sections Anemonanthea, Rivularidium, Pulsatilloides, Eriocephalus, Anemonidium, and Homalocarpus. The various subcategories which he established and the species therein which are mentioned in this study will be listed later.

In his revision of *Anemone*, 1937, Juzepczuk (16) has followed Ulbrich's classification in general except that he raised *Hepatica* to generic status, recognized each of the sections as subgenera, and made several revisions within some of the subcategories.

Janchen (14) accepted Ulbrich's classification except that he recognized the independent status of *Hepatica*. He confirmed the relationship among the genera formerly included in *Anemone* s. lat. by placing them all in the subtribe Anemoninae.

However, these revisions have not been generally accepted and some taxonomists continue to interpret the genus *Anemone* in the liberal manner of Prantl (28). In particular, recognition of the genera *Pulsatilla* and *Hepatica* is questioned and thus the limits of the genus *Anemone* remain unsettled.

A recent monograph of 1957 by Aichele and Schwegler (1) includes extensive revisions within the genus *Pulsatilla* Mill.

Cytological data have been reviewed by Gregory (12), the more extensive papers being those of Langlet (19, 20) and Moffett (26). These papers established the chromosome numbers of many species of *Anemone* and recognized two series based on 7 and 8 chromosomes respectively. From his cytological studies Langlet (19) associated species of *Anemone* with the forms characterized by the large *Ranunculus* type of chromosome as contrasted with the small *Thalictrum* type characteristic of certain other genera of the family Ranunculaceae Juss. Moffett (26) described the karyotype common to species with 16 chromosomes as consisting of four pairs with median or submedian constrictions, one pair in which the short arm is about one-third of the length of the long arm, and three pairs with subterminal constrictions, and the karyotype of *Hepatica*, with 14 large chromosomes, as consisting of six pairs with median or near-median constrictions and one pair with subterminal constrictions. Moffett interpreted the difference between the 14- and 16-chromosome karyotypes as arising from fusion.

Kurita (17, 18) studied the chromosome morphology of 11 species of *Anemone* s. str., two species of *Pulsatilla*, and one species and two varieties of *Hepatica*. He recognized a 7-chromosome series represented by *A. flaccida* with karyotypes essentially similar to those of *Hepatica* described by Moffett (26) and an 8-chromosome series represented by *A. nipponica* with karyotypes similar to those of 16-chromosome species also described by Moffett. In addition, size differences among the short arms of the three pairs of chromosomes with subterminal constrictions were noted. Kurita's 7-chromosome series includes *A. keiskeana*, *A. flaccida*, *A. tagawae*, *A. narcissiflora*, and *Hepatica nobilis* and three varieties, and his 8-chromosome series, *A. coronaria*, *A. hybrida*, *A. nikoensis*, *A. nipponica*, *A. pseudo-altaica*, *A. raddeana* var. *integra*, *A. stolonifera*, *Pulsatilla cernua*, and *P. nipponica*. While Kurita (18) accepted Moffett's interpretation (26) of the difference between the 7- and 8-chromosome series as arising from fusion he considered this too simple an explanation to account for the differences between the karyotypes of *A. flaccida* and *A. nipponica*.

Many papers reporting chromosome numbers only are not reviewed here and can be found by reference to the various lists compiled by Tischler (29), Darlington and Wylie (8), and others.

Materials and Methods

Seeds and plants from the wild supplemented by materials from commercial firms and botanical gardens have been established in the gardens and greenhouses of the University of Toronto.

Root tips of seedlings and plants were pretreated in .1% colchicine for 1 hour, fixed in absolute alcohol and acetic acid 3:1 for half an hour to an hour, stained according to the Feulgen schedule, squashed in aceto-orcein, and made permanent by the dry ice method of Conger and Fairchild (6).

Two to 10 suitable plates from each species were drawn with the aid of a camera lucida at a magnification of 5000. Chromosome arms were measured in the drawings without inclusion of the centromere region, the long arms by using a map measuring device, the very short arms, with a straight ruler. Measurements are given in micra to one decimal place. The chromosomes of each plate were arranged in order of increasing length from left to right with the centromeres in a straight line. This arrangement usually resulted in the short arms also forming an orderly series of increasing length and chromosomes falling into properly associated pairs. In the 8-chromosome series, the pairs of chromosomes with subterminal constrictions can be identified readily by size differences in their short arms. With strict adherence to the procedure outlined, it was sometimes obvious that these were mispaired. In such cases an adjustment was made to bring them into proper association. Similarly among the longest chromosomes, if several were nearly equal in length, arm ratios were used as an aid to identification and arrangement.

Averages of the measurements which are given in the tables were used in the preparation of the idiograms. In view of the diversity of the material

examined and the fact that comparisons have been based on conspicuous features of chromosome morphology, no mathematical treatment has been attempted.

Comparisons are based on relationships between total lengths and arm ratios of the various pairs, the size and position of satellites, and the pattern formed by the chromosomes when arranged according to the method described above.

Unless otherwise specified, voucher specimens are in the herbarium of the author from which they will be transferred to the herbarium of the University of Toronto at an appropriate time. Accession numbers identify the materials in the herbarium, gardens, and greenhouses.

The abbreviation, D.A.O., refers to the herbarium of the Canada Department of Agriculture, Ottawa.

Descriptions

In the system outlined the chromosome arrangements follow particular patterns. Karyotypes with 16 chromosomes have three pairs with subterminal constrictions at the left, each pair identified by short arms of different lengths on which satellites may or may not be present. A pair with submedian constrictions appears in the fourth position followed by four pairs with median or near-median constrictions which form a more or less graded series.

Karyotypes with 14 chromosomes have a single pair with subterminal constrictions at the left followed by six pairs with median or near-median constrictions which form a series of increasing length.

Since karyotypes of species studied follow one or the other of these two patterns, the descriptions will be limited to variations within the patterns. These observations can be supplemented by references to the figures, tables, idiograms, and plate.

The following data appear after the name of the species: accession numbers, sources of material, collectors other than the author, plant accessions as distinguished from populations raised from seeds, number of plates from which drawings were made, figure and table numbers, and chromosome numbers found by other investigators.

The presentation of data follows essentially Ulbrich's system of classification for *Anemone* (30) except that the generic status of *Hepatica* has been recognized. The following outline includes only the species which are mentioned in the text.

Genus *Anemone*
Section ANEMONANTHEA
Subsection Sylvia
Series Hylalectryon
A. keiskeana, A. nikoensis, A. pseudo-altaica, A. quinquefolia, A. raddeana var. *integra*
Subsection Stolonifera
A. baicalensis, A. flaccida, A. stolonifera

Section RIVULARIDIUM
Series Rivularis
A. antucensis, A. leveillei, A. rivularis, A. tenuicaulis
Series Crassifolia
A. crassifolia
Series Richardsonia
A. richardsonii

Section ERIOCEPHALUS
Subsection Longistylae
Series Baldensis
A. drummondii
Series Oriba
A. coronaria, A. hortensis, A. palmata, A. pavonina
Series Parviflora
A. parviflora
Subsection Brevistylae
Series Anemonospermos
A. hupehensis, A. rupicola, A. silvestris, A. tomentosa, A. vitifolia
Series Virginiana
A. cylindrica, A. riparia, A. virginiana
Series Multifida
1. Etuberosa
A. multifida
2. Tuberosa
A. caroliniana
Section ANEMONIDIUM
A. canadensis
Section HOMALOCARPUS
A. fasciculata, A. narcissiflora

Species of the genus *Pulsatilla* have been classified according to the monograph of Aichele and Schwegler (1).

Genus *Pulsatilla*
Section PULSATILLA
Subsection Pratenses
P. pratensis
Subsection Patentes
P. nuttalliana ssp. *nuttalliana*
Section SEMICAMPANARIA
P. cernua
Section PREONANTHUS
P. occidentalis
Section PREONANTHOPSIS
P. nipponica

Genus *Anemone* L.
Section ANEMONANTHEA DC.
Subsection Sylvia Gaud.
Series Hylalectryon Irmisch.

A. quinquefolia L. 611, near Grand Bend, SE. shore of Lake Huron, Ont., Sept. 25, 1958. Two plants, three plates. Figs. 1A, 11. Table I. $2n = 32$, Löve (24).

The chromosome number of 32, in agreement with that reported by Löve, was also found in a collection from Rattlesnake Point, Halton Co., Sept. 7, 1955. The assignment of chromosome pairs to respective sets is purely arbitrary and is intended only to facilitate comparison of chromosomes in comparable positions. Evidence that two distinct sets are involved can be found in examination of the short-armed pairs where two like sets cannot be identified, there being three pairs with satellites and one pair in the third position with long arms much shorter than those of the other pairs. Arm ratios of about 1:2 are found in the chromosome pairs in the fourth position. Of the seven North American species recognized by Fernald (9), two from the east and five from the Rocky Mountains, *A. quinquefolia* only has been studied cytologically.

Section RIVULARIDIUM Jancz.
Series Rivularis Ulbr.

A. rivularis Buch. Ham. 141, Botanical Garden, Freiburg, Germany, 1955. One population, three plates. Figs. 1B, 12. Table I. $2n = 16$, Langlet (20). *A. rivularis* var. *barbulata*, $2n = 16$, Gregory (12).

The chromosome count of 16 is in agreement with earlier reports. The fourth pair of chromosomes with arm ratios of 1:1.4 are unusually small, being similar to the third pair in size rather than to the fifth pair. The

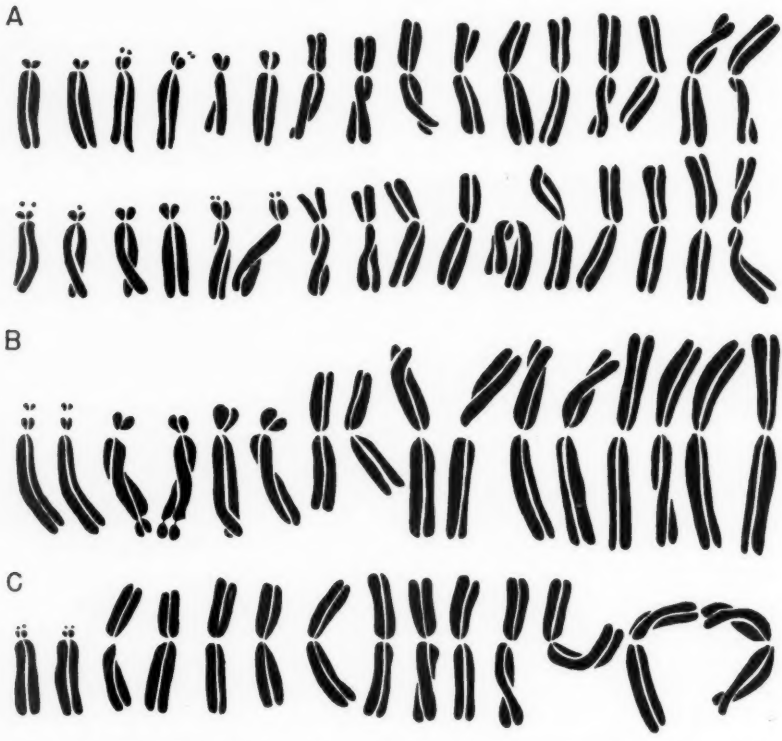

FIG. 1. Drawings of metaphase chromosomes of *Anemone*. ×2500. A, *A. quinquefolia*. B, *A. rivularis*. C, *A. richardsonii*.

second pair have pronounced near-terminal constrictions in their long arms. In addition to population 141, six others of varying origins have been assigned to the series Rivularis because of the distinctive character of their achenes. They have been examined but not included in the figures or tables since they have not flowered. In the vegetative state they are indistinguishable from the above but differ considerably in cytological details. Four of these have constrictions in the long arms of the second pair of chromosomes as in population 141 but the other two have such constrictions in the long arms of the first pairs. In one population of the second group the fourth pairs have arm ratios of 1:1.4 like those of population 141 but these pairs are similar in size to the fifth rather than the third pairs. In the remaining populations of both groups, the chromosome pairs in the fourth position are similar in size to those of the fifth and have arm ratios of about 1:1.6. This is sufficient to show that considerable diversity exists in the series Rivularis. However, the variation in the karyotypes studied may be due in part to the fact that only materials from botanical gardens where hybridization may have

TABLE I*

	Chromosome																			
	I			II			III			IV			V			VI			VII			VIII
Section Anemonanthea																				
A. quinquefolia, $2n = 32$		.7			1.0–.2			1.1			2.4			3.4			3.8			4.4			4.7
		5.5			5.9			5.1			4.8			4.3			5.0			4.8			5.2
	7.8	6.2		5.9	7.1		4.6	6.2		2.0	7.2		1.3	7.7		1.3	8.8		1.1	9.2		1.1	9.9
		.8–.2			1.1			1.5–.2			2.5			3.6			4.3			4.5			5.4
		5.8			5.8			6.1			4.9			4.6			4.5			5.0			5.6
	7.2	6.8		5.3	6.9		4.0	7.8		1.9	7.4		1.3	8.2		1.0	8.8		1.1	9.5		1.0	11.0
A. flaccida (from Kurita), $2n = 14$		.5–.4			5.0			5.4			5.9			6.3			6.8			6.9			
		7.3			5.7			6.3			6.7			6.8			6.8			7.1			
	14.6	8.2		1.1	10.7		1.2	11.7		1.1	12.6		1.1	13.1		1.0	13.6		1.0	14.0			
Section Rivularidium																							
A. rivularis, $2n = 16$		.7–.3			1.1			1.5			3.3			5.2			5.5			5.6			6.0
		5.9			6.1			6.0			4.5			6.0			6.1			6.6			6.6
	8.4	6.9		5.5	7.2		4.0	7.5		1.4	7.8		1.2	11.2		1.1	11.6		1.2	12.2		1.1	12.6
A. richardsonii, $2n = 14$		.5–.3			3.3			4.0			4.4			4.4			4.0			5.4			
		5.1			4.9			4.6			5.0			5.0			6.0			5.9			
	10.0	5.9		1.5	8.2		1.2	8.6		1.1	9.4		1.1	9.4		1.5	10.0		1.1	11.3			

*Chromosome measurements are placed to the right in each column as follows: first measurement refers to the short arms; the second, to the long arms; and the third, to the totals. Measurements of satellites are to the right of the appropriate arm. Arm ratios appear at the left.

occurred were available and types not present under natural conditions may have been investigated. Or, it may be possible that a greater diversity exists under natural conditions than has been recognized taxonomically.

Series Richardsonia Ulbr.

A. richardsonii Hook. 267, from near Anchorage, Alaska, Mrs. Wm. Manley, July, 1956. One plant, three plates. Figs. 1C, 6, 13. Table I. $2n = 14$, Bormann and Beatty (3).

The chromosome count of 14 as reported above is confirmed. The presence of the two chromosome pairs with arm ratios of about 1:1.5 in the second and sixth positions respectively is an unusual feature of the karyotype of this species.

Section Eriocephalus Hook. and Thoms.
Subsection 1. Longistylae Ulbr.
Series 1. Baldensis Ulbr.

A. drummondii Wats.

Plants with 32 and 48 chromosomes collected in the wild and others with 32 chromosomes grown from seeds have been obtained from the Rocky Mountains under this name but will not be discussed at this time since they have not flowered.

Series 2. Oriba Adans.

A. palmata L. 449, Hortus Botanicus, Coimbra, Portugal, 1957. One population, three plates. Figs. 2A, 12. Table II. $2n = 32$, Langlet (20).

Sixteen chromosomes were found in this material rather than 32 as reported by Langlet. The karyotype of this species differs from the general pattern in several respects. The third and fourth pairs appear to be identical except that the short arms of the fourth pairs have pointed tips. The arms of the fifth pair are nearly equal in length, with the short arms exceeding those of the pairs which follow in the series. Also noteworthy is the striking contrast in size between the satellites on the short arms of the first and second pairs.

A. pavonina Lam. var. *ocellata* (Moggr.) Bowles. 561, received as *A. fulgens* from the American Rock Garden Society Seed Exchange, 1957. One population, three plates. Figs. 2B, 12. Table II. *A. fulgens*, $2n = 16$, Moffett (26).

Sixteen chromosomes were present in this material in agreement with the report for the related taxon. Arm ratios of 1:1.6 are present in the fourth pairs. Neither the short nor the long arms of the first three pairs differ much in size. The short arms of the first two pairs have satellites. The fifth pair is considerably longer than the fourth pair.

The relationships among *A. pavonina*, *A. hortensis*, and *A.* × *fulgens* are explained by Bowles (4).

A. coronaria L. 431, "St. Brigid" from Rex Pearce Seed Co., 1957; 440, "de Caen" various, 437, "de Caen" rouge ponceau, 438, "chrysanthemiflora", all from Vilmorin-Andrieux, Paris, 1957. Four populations, 12 plates. Figs. 2C, 12. Table II. $2n = 16$, Guinochet (13).

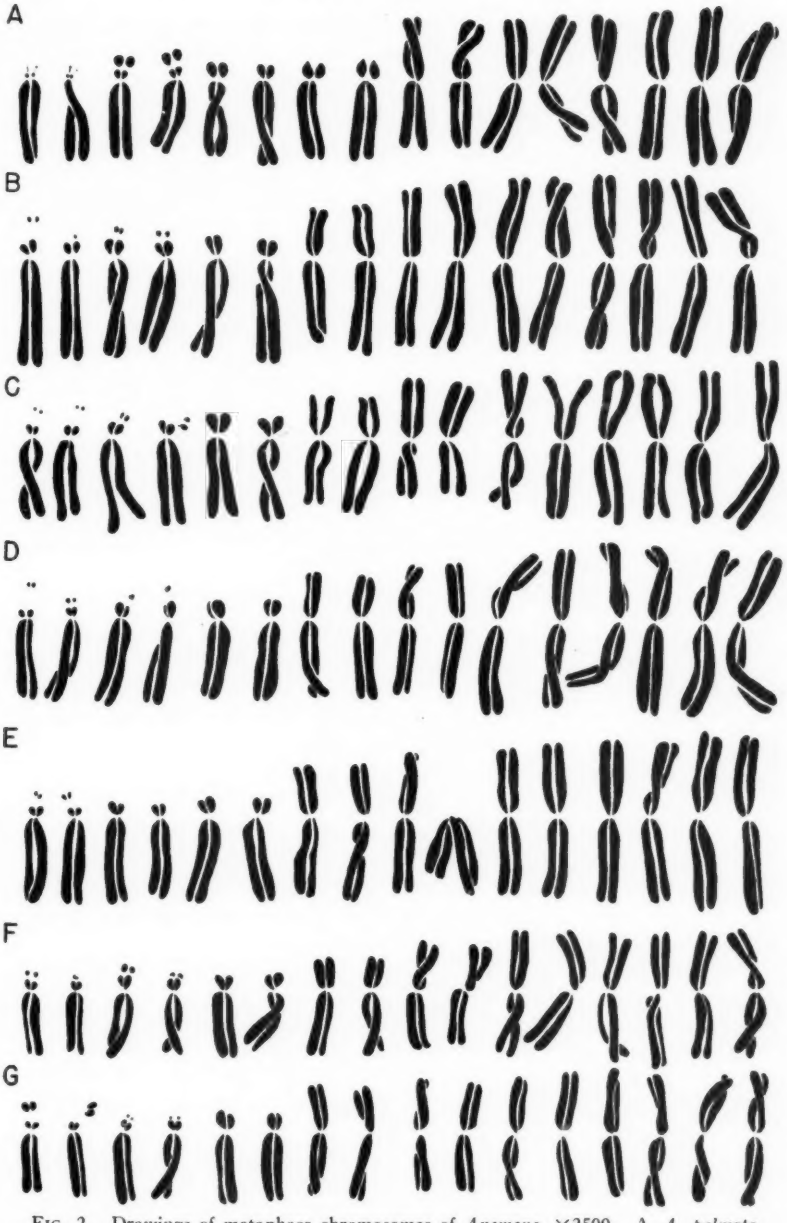

FIG. 2. Drawings of metaphase chromosomes of *Anemone*. ×2500. A, *A. palmata*. B, *A. pavonina*. C, *A. coronaria*. D, *A. parviflora*. E, *A hupehensis*. F, *A. silvestris*. G, *A. rupicola*.

The chromosome count of 16 is in agreement with the previous report. The karyotype of this species resembles that of *A. pavonina*, differing chiefly in the fourth and fifth pairs being about equal in size. Arm ratios of 1:1.6 are present in the fourth pair.

Series 3. Parviflora Ulbr.

A. parviflora Michx. 490, Churchill, Man., seeds, Mrs. R. M. Beckett, 1957; 602, Seven-Mile Flat, Clearwater Forest Reserve, Alta., plants, C. C. Heimburger, June 6, 1958. One population, two plants, five plates. Figs. 2D, 11. Table II. $2n = 16$, Langlet (20), Moffett (26); $2n = 14$, Bormann and Beatty (3).

Plants from both localities have 16 chromosomes. This is in agreement with earlier reports rather than with the more recent one of Bormann and Beatty (3). Complements from plants in the seedling population from Churchill appeared to have centromeres in less median positions in the longer chromosomes than was observed in plants from Clearwater Forest Reserve. Perhaps this may be accounted for by the rather inferior quality of the preparations. Chromosome IV has arm ratios of about 1:2.

Subsection 2. Brevistylae Ulbr.
Series 4. Anemonospermos DC. s. ampl.

A. hupehensis Lam. 419, Istituto e Orto Botanico, Rome, 1958; 443, Botanical Garden, Cologne, Germany, 1957; 483, source unknown. Three populations, nine plates. Figs. 2E, 12. Table II. $2n = 16$, Langlet (19). *A. vitifolia*, $2n = 16$, Moffett (26), Langlet (20); $n = 8$, Gajewski (11). *A. japonica* Sieb. and Zucc., $2n = 16$, Moffett (26), Gregory (12). *A. nipponica* Mer. (= *A. hupehensis* Lam. var. *japonica* (Thunb.) Bowles and Stearn), $2n = 16$, Kurita (17, 18). *A. hybrida* Godron, $2n = 16$, Kurita (18).

The various taxa listed are all part of a horticultural complex involving *A. vitifolia*, *A. tomentosa*, and *A. hupehensis* which has been described by Bowles and Stearn (5).

Agreement can be noted between the descriptions of the karyotype of *A. nipponica* (18) and that of *A. hupehensis* given here in the presence of satellites on the short arms of the first pairs, the short arms of the first and second pairs being nearly alike in size and in the short arms of the third pairs being almost twice as long as those of the second pairs. The arm ratio of 1:1.6 is found in chromosome IV. The karyotypes of these taxa differ particularly in respect to size of the chromosomes and in the arm ratios of the second and third pairs (Table II). More variation may be found as karyotypes of taxa in the complex become better known.

A. silvestris L. 154, St. Gallen, Switzerland, 1955; 445, Botanical Garden, Cologne, 1957; 434, Alpenglow Gardens, Westminster, B.C., 1957. Three populations, 10 plates. Figs. 2F, 11. Table II. $2n = 16$, Langlet (19), Moffett (26); $n = 8$, Gajewski (10, 11).

Sixteen chromosomes were found to be present, as has been previously reported. The fourth chromosome has an arm ratio of 1:2 and in this respect differs from other species in the series Anemonospermos. The fourth and fifth pairs are about equal in length. Measurements obtained by Gajewski (10) without pretreatment are as follows: the first three pairs, 4.5 μ; the fourth pair, 5.5 (2+3.5) μ; the fifth and sixth pairs, 6 (3+3) μ; the seventh pair, 7 (3.5+3.5) μ; and the eighth pair, 9 (4.5+4.5) μ. The measurements of the longest pair are in agreement with those obtained in the present study (Table II). The original sources of the materials at hand are not known but in view of the wide distribution of this species in Europe and Asia and the taxonomic diversity described, some cytological differences might be expected. The main difference noted in the present study is the presence of small satellites on the short arms of the third chromosome pair of population 445 apparently lacking in the other two populations examined.

A. rupicola Cambess. 488, Kew Gardens, 1957. One population from seeds of one plant, three plates. Figs. 2G, 12. Table II. $2n = 32$, Langlet (19), Moffett (26).

Sixteen chromosomes only were found in the material studied rather than 32 as reported by Langlet and Moffett. The karyotype of this species resembles that of *A. hupehensis* in having arm ratios of 1:1.6 in the fourth pair and those of two populations of *A. silvestris* in having satellites on the short arms of the first two pairs.

Series 5. Virginiana Ulbr.

A. cylindrica Gray. 95, Haldimand Tp., Ont., Aug. 7, 1955; 173, Victoria Beach, Man., J. Morgan-Jones, 1955; 321, Oliphant, Bruce Co., Ont., Sept. 30, 1956; 323, Ganaraska Forest, Northumberland Co., Ont., C. C. Heimburger, Sept. 27, 1956; 359, Head Lake, Victoria Co., Ont., K. Rothfels, Nov. 18, 1956. Five populations, 10 plates. Figs. 3A, 9, 11. Table II. $2n = 16$, Dahl (7); $n = 8$, Gregory (12).

The chromosome number of 16 found in this study is in agreement with previous reports. The position of the satellites differs from those already described in that they are present on the short arms of the third pair of chromosomes. The fourth pairs have arm ratios of 1:2 and are about equal in size to the fifth pair, features also found in *A. silvestris*. *A. cylindrica* is part of the controversial *A. virginiana–riparia–cylindrica* complex which is currently being investigated and was chosen to represent the group since it is the taxon most easily recognized. Others of the complex also have arm ratios of 1:2 in the fourth pairs.

Series 6. Multifida Ulbr.

1. Etuberosa Ulbr.

A. multifida Poir. 93, Portage Bay, Manitoulin Island, Ont., July 24, 1955; 211, Kindersley District, Sask., Jenkins 6230, July 19, 1955. (D.A.O.); 213, Fort Simpson, Northwest Territories, W. J. Cody & J. M. Matte, 9220,

Aug. 5, 1955. (D.A.O.); 389, Montana, Mrs. Olga Johnson, 1956; 495, Churchill, Man., Mrs. R. M. Beckett, 1957; 592, Parque Nac. Nabeiel, Huapé, Argentine. José Diem, 1958. Eight populations, 10 plates. Figs. 3B, 8, 11. Table II. $2n = 32$, Moffett (26), Langlet (19), Gajewski (10).

Thirty-two chromosomes were found in root tips of all eight populations. Marked differences in size between some of the chromosome pairs indicate that two sets are involved. The chromosome pairs have been assigned to different sets on the basis of size relationships and contrasting arm ratios in the pairs with submedian constrictions. The three small pairs with subterminal constrictions, the small pair with arm ratios of 1:1.6, and a fifth pair related to the last in size are considered to belong to one set. The three corresponding large pairs with subterminal constrictions, the pair with arm ratios of 1:2, and the two longest pairs are considered as belonging to the second set. Five remaining pairs of overlapping size have been assigned arbitrarily. When this separation of pairs into two groups is made, the first group resembles complements of the genus *Pulsatilla*, particularly in the section Pulsatilla. The chromosomes of the second group resemble complements of *A. silvestris* or *A. cylindrica* in arm ratios and relative size. Support for recognition of affinities between one of the chromosome sets of *A. multifida* and complements of *A. silvestris* and the *A. virginiana–riparia–cylindrica* complex can be found in the analysis of chromosome pairing in the hybrids between *A. multifida* and the above diploid taxa (10, 11, and current investigations).

No significant differences were found between the chromosome complements of *A. multifida* from widely separated areas of its range. Hybridization experiments and subsequent analysis of chromosome pairing in the hybrids can be expected to reveal differences not detectable by the techniques employed in the present study.

2. Tuberosa Ulbr.

A. caroliniana Walt. 537, American Perennial Gardens, Garden City, Michigan, 1958. One population, two plates. Figs. 3C, 11. Table II. $n = 8$, Moffett (26).

The chromosome number was found to be 16 which is in agreement with Moffett's report. Attention is called to the arm ratio of about 1:2 in the fourth chromosome pair, the rather large third chromosome pair, and the large satellites on the short arms of the first pair.

Section Anemonidium Spach.

A. canadensis L. 174, Victoria Beach, Man., J. Morgan-Jones, 1955. One population, three plates. Figs. 4A, 13. Table III. $2n = 14$, Langlet (20); $2n = 16$, Guinochet (13).

The chromosome number proved to be 14 in agreement with that reported by Langlet. Seven pairs of chromosomes were found at meiosis in pollen mother cells of plants from Peel and York Counties, Ontario, June, 1955.

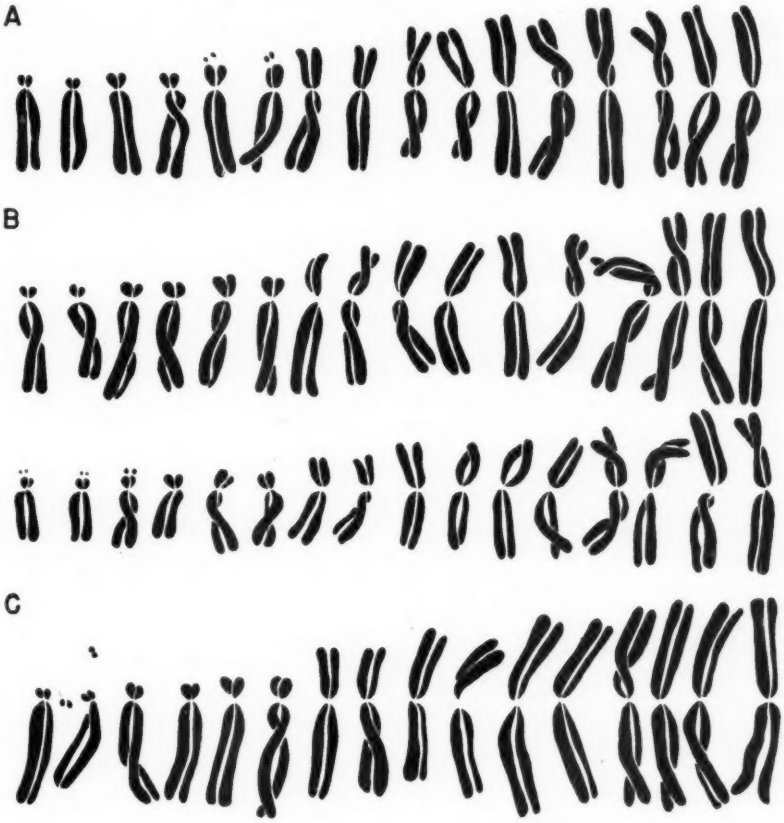

FIG. 3. Drawings of metaphase chromosomes of *Anemone*. ×2500. A, *A. cylindrica*. B, *A. multifida*. C, *A. caroliniana*.

The count recorded by Guinochet was included in Gregory's survey of cytological work in the Ranunculaceae (12) and has been circulated more widely than the earlier report of Langlet.

The second pair of chromosomes have arm ratios of 1:1.6.

Section HOMALOCARPUS DC. emend.

A. fasciculata L. 400, Institutum Botanicum nom. V. L. Komarovii Academiae Scientiarum URSS, Leningrad, 1956. One plant from seeds, two plates. Figs. 4B, 13, Table III. *A. narcissiflora* L., $2n = 14$, Langlet (21), Kurita (17, 18).

This plant has not flowered and the identification is that under which it was received. It can be assigned to this section on account of the characteristic achenes which resemble those of *A. canadensis* but are smooth and have

TABLE II*

	Chromosome															
	I		II		III		IV		V		VI		VII		VIII	
Section Eriocephalus																
A. palmata		.2–.1		.2–.8		1.0		1.0		3.9		3.8		3.9		4.2
		4.9		4.9		4.7		4.7		4.0		4.3		4.5		4.8
	24.5	5.2	24.5	5.9	4.7	5.7	4.7	5.7	1.0	7.9	1.1	8.1	1.2	8.4	1.1	9.0
A. pavonina		.8–.3		1.0–.3		1.2		2.9		4.3		4.8		4.9		5.1
var. *ocellata*		5.9		5.9		5.8		4.9		4.9		5.2		5.3		5.6
	7.4	7.0	5.9	7.2	4.8	7.0	1.7	7.8	1.1	9.2	1.1	10.0	1.1	10.2	1.1	10.7
A. coronaria		.7–.2		.9–.3		1.2		2.7		3.7		4.2		4.6		4.8
		5.1		5.2		4.9		4.4		3.9		4.6		5.0		5.2
	7.3	6.0	5.8	6.4	4.1	6.1	1.6	7.1	1.1	7.6	1.1	8.8	1.1	9.6	1.1	10.0
A. parviflora		.6–.4		.8		1.0		2.0		2.6		3.5		3.7		3.8
		4.0		4.1		4.1		3.8		3.7		4.2		4.5		4.6
	6.6	5.0	5.1	4.9	4.1	5.1	1.9	5.8	1.4	6.3	1.2	7.7	1.2	8.2	1.2	8.4
A. nipponica		.4–.1		.7		1.2		2.6		3.5		3.5		3.7		3.9
(from Kurita)		4.1		4.1		4.2		4.0		3.5		3.9		4.2		4.3
	10.0	4.6	5.9	4.8	3.5	5.4	1.5	6.6	1.0	7.0	1.1	7.4	1.1	7.9	1.1	8.2
A. hupehensis		.6–.3		.7		1.2		3.2		4.1		4.5		4.9		5.0
		5.4		5.4		5.6		5.3		4.7		5.0		5.6		5.9
	9.0	6.3	7.7	6.1	4.7	6.8	1.7	8.5	1.1	8.8	1.1	9.5	1.1	10.5	1.2	10.9
A. silvestris		.6–.2		.9–.3		1.1		2.2		3.1		3.9		4.1		4.3
		4.3		4.6		4.5		4.4		3.7		4.4		4.7		4.9
	7.1	5.1	5.1	5.8	4.1	5.6	2.0	6.6	1.2	6.8	1.1	8.3	1.1	8.8	1.1	9.2
A. rupicola		.4–.7		.5–.2		1.0		2.6		3.2		3.6		3.8		3.9
		4.2		4.3		4.2		4.1		3.5		4.1		4.2		4.5
	10.5	5.3	8.6	5.0	4.2	5.2	1.6	6.7	1.1	6.7	1.1	7.7	1.1	8.0	1.1	8.4
A. cylindrica		.7		1.0		1.4–.2		2.4		3.5		4.0		4.8		5.0
		5.0		5.1		5.2		5.0		4.2		5.3		5.6		5.9
	7.1	5.7	5.1	6.1	3.7	6.8	2.1	7.4	1.2	7.7	1.3	9.3	1.2	10.4	1.2	10.9
A. multifida		.7		1.0		1.3		2.4		3.1		3.3		4.9		5.1
		5.0		5.3		5.4		5.0		4.1		4.5		5.8		5.9
	7.1	5.7	5.3	6.3	4.2	6.7	2.1	7.4	1.3	7.2	1.4	7.8	1.2	10.7	1.2	11.0
		.6–.2		.8–.3		1.0		1.9		2.6		3.5		3.8		4.4
		3.3		3.3		3.5		3.2		3.3		3.9		4.3		4.7
	5.5	4.1	4.1	4.4	3.5	4.5	1.7	5.1	1.3	5.9	1.1	7.4	1.1	8.1	1.1	9.1
A. caroliniana		.5–.6		1.1		1.5		2.9		4.5		5.5		5.5		5.9
		6.1		6.0		6.6		5.5		5.0		6.1		6.3		6.7
	12.2	7.2	5.5	7.1	4.4	8.1	1.9	8.4	1.1	9.5	1.1	11.6	1.1	11.8	1.1	12.6

*See footnote, Table I.

thin rather than corky wings (15). Its distinctive karyotype is in agreement with that described and figured by Kurita (18) for *A. narcissiflora* with which it is allied. The arm ratio of 1:1.4 in the second pair should be noted and also the satellites on the short arm. The chromosomes of this plant are larger than any yet found in *Anemone*. Satellites are also present on the short arms of the first pair of chromosomes, which differ in this respect from

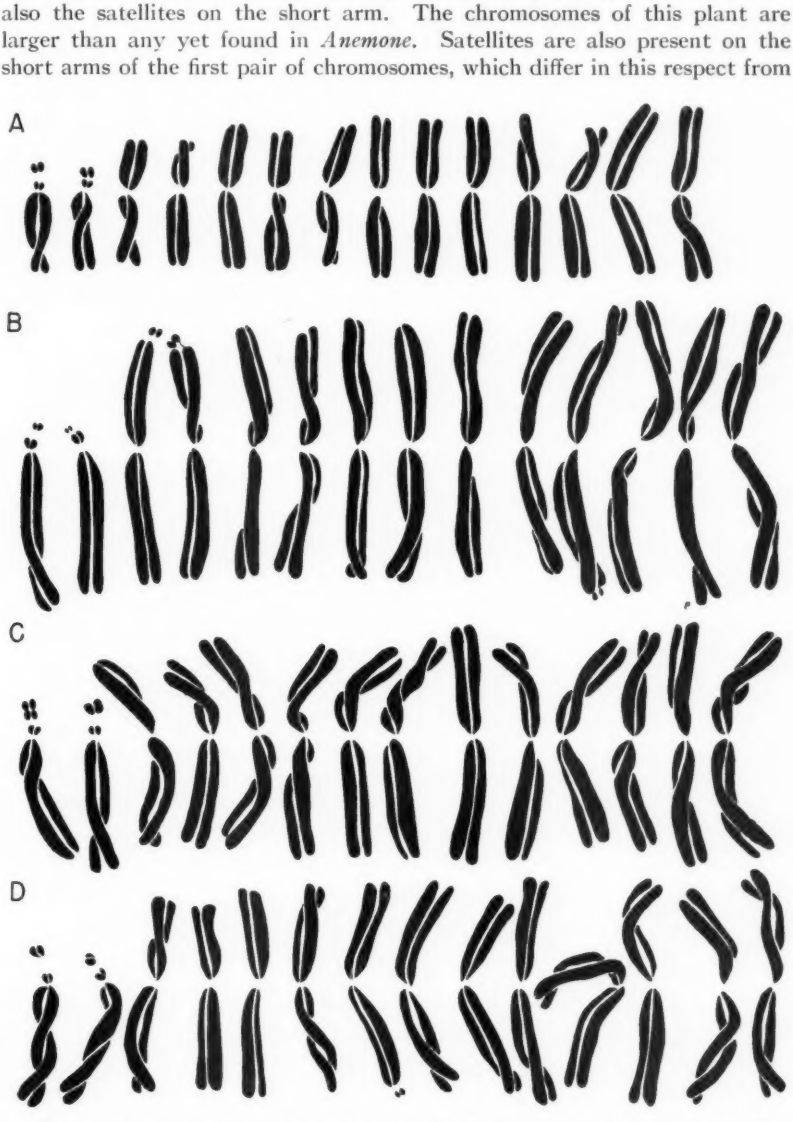

FIG. 4. Drawings of metaphase chromosomes of *Anemone*, A, B; *Hepatica*, C, D. ×2500. A, *A. canadensis*. B, *A. fasciculata*. C, *H. acutiloba*. D, *H. americana*.

*See footnote, Table I.

TABLE III*

	Chromosome													
	I		II		III		IV		V		VI		VII	
Section Anemonidium														
A. canadensis		.5–.7		2.8		3.8		4.0		3.8		4.5		4.8
		5.0		4.4		4.1		4.3		4.9		4.9		5.0
	10	6.2	1.6	7.2	1.1	7.9	1.1	8.3	1.3	8.7	1.1	9.4	1.0	9.8
Section Homalocarpus														
A. fasciculata		.8–.4		6.1–.5		7.5		7.6		8.0		8.6		9.1
		9.3		8.5		7.8		8.6		8.4		9.4		9.7
	11.6	10.5	1.4	15.1	1.0	15.3	1.1	16.2	1.1	16.4	1.1	18.0	1.1	18.8
Genus *Hepatica*														
H. nobilis var. *japonica* (from Kurita)		.6–.7		5.4		6.2		6.2		6.7		6.9		7.6
		8.6		7.0		7.3		7.7		7.8		8.2		8.1
	14.3	9.9	1.3	12.4	1.2	13.5	1.2	13.9	1.2	14.5	1.2	15.1	1.1	15.7
H. americana		.6–.7		5.3		5.8		7.0		6.3		7.2		7.6
		8.5		7.1		7.5		7.3		8.2		7.9		8.1
	14.1	9.8	1.4	12.4	1.3	13.3	1.0	14.3	1.3	14.5	1.1	15.1	1.1	15.7
H. acutiloba		.6–.8		4.9		5.6		6.2		6.2		6.6		7.0
		8.0		6.8		7.0		6.8		7.3		7.1		7.5
	13.3	9.4	1.4	11.7	1.3	12.6	1.1	13.0	1.2	13.5	1.1	13.7	1.1	14.5

*See footnote, Table I.

Kurita's description (18). Kurita (17) gave measurements for the longest and shortest chromosomes of *A. narcissiflora* as 15.4 and 9 μ respectively, which are shorter than those obtained in this study (Table III).

Genus *Hepatica* Mill.

Hepatica americana (DC.) Ker. 617, near Maple, York Co., Ont., Sept. 21, 1958. Four plants, six plates. Figs. 4D, 13. Table III.

The chromosome number of 14 is reported for the first time. Noteworthy features include the large size of the chromosomes, the long arms of the first pair of chromosomes being the longest of all the pairs, and the arm ratio of 1:1.4 in the second pair. Measurements of chromosomes of *H. nobilis* var. *japonica* published by Kurita (18) are almost identical with those for *H. americana* of this study. Arm ratios differ somewhat.

H. acutiloba DC. 78, Rattlesnake Point, Halton Co., Ont., Sept. 7, 1955; 618, near Maple, York Co., Ont., Sept. 21, 1958. Three plants, four plates. Figs. 4C, 7, 13. Table III. $2n = 14$, Langlet (20).

Fourteen chromosomes were found in the root tips in agreement with the count reported by Langlet. The karyotype is essentially similar to that of the preceding species.

Genus *Pulsatilla* Mill.
Section Pulsatilla (DC.) Aichele and Schwegler.
Subsection Pratenses Juz.

P. pratensis (L.) Mill. 144, Botanical Garden, Freiburg, Germany, 1955. One population, three plates. Figs. 5B, 12. Table IV. $2n = 16$, Langlet (19), Böcher (2).

The chromosome count of 16 is in agreement with previous reports. Two pairs of satellites were noted by Böcher (2) and these have been found to be present on the short arms of the first two pairs in the present material. Attention is directed to the somewhat smaller size of the chromosomes in comparison with those of known species of *Anemone*, the small size of the first pair and arm ratios of 1:1.6 in the fourth pair.

Subsection Patentes Juz.

P. nuttalliana (DC.) Bercht. Presl ssp. *nuttalliana*. 227, Montana, Fred Rose, 1955; 538, Big Horn Mountains, Wyoming, Fred Rose, 1957; 575, Eskimo Lakes, Mackenzie District, Cody & Ferguson 10449, (D.A.O.). Three populations, 10 plates. Figs. 5A, 10, 12. Table IV. $2n = 16$, Bormann and Beatty (3); $2n = 32$, Löve (25).

These populations and population 393 from Alaska, which was examined but not drawn, all have 16 chromosomes as reported by Bormann and Beatty. Plants examined by Löve came from Manitoba; I have examined no material from this region. The karyotype of this species is indistinguishable from the preceding and the same noteworthy features are present in both.

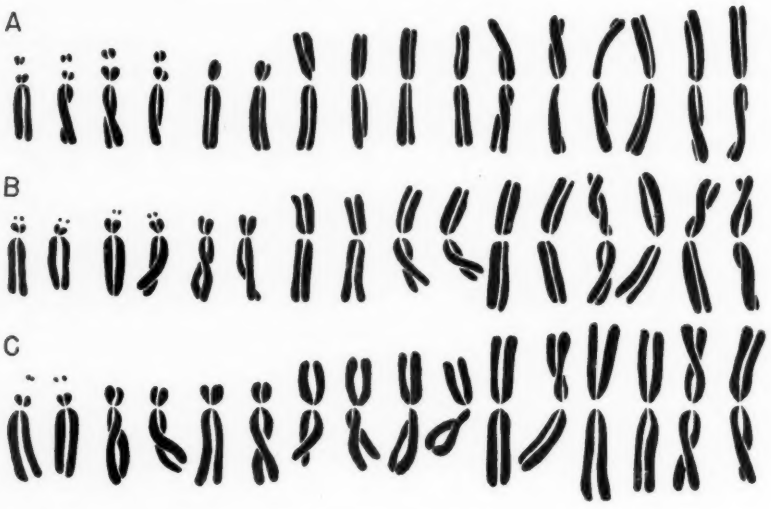

FIG. 5. Drawings of metaphase chromosomes of *Pulsatilla*. ×2500. A, *P. nuttalliana* ssp. B, *P. pratensis*. C, *P. occidentalis*.

Section PREONANTHUS Ehrh.

P. occidentalis (Wats.) Freyn. 394, Washington, Mrs. Birdie Padavich, 1957, through the American Rock Garden Society Seed Exchange. One population, three plates. Figs. 5C, 12. Table IV.

Root tips of seedlings from the following populations were examined but not included in the above: 621, Sunshine and 627, Lake Agnes, Banff National Park, Alta., A. E. Porsild, 1958; 628, Kananaskis, Alta., E. H. Moss, 1958.

The 16 chromosomes of this species are reported for the first time. The karyotype is unusual in that the short arms of the second and third pairs are almost equal in length and the short arms of the first three pairs are large in comparison with the long arms. The arm ratio of 1:1.4 in the fourth chromosome pair is not common among species with 16 chromosomes. Satellites were noted on the first pairs of chromosomes in population 394. In the remaining populations small satellites were observed also on the short arms of the second pairs. Satellites of unequal size occur in population 628.

Herbarium specimens of *P. nuttalliana* and *P. occidentalis* consist of seedlings only at the present time. Seeds germinate readily and seedlings grow well until the hot humid weather of midsummer when most die.

FIGS. 6–10. Metaphase complements. Fig. 6. *Anemone richardsonii*. Fig. 7. *Hepatica acutiloba*. Fig. 8. *Anemone multifida*. Fig. 9. *Anemone cylindrica*. Fig. 10. *Pulsatilla nuttalliana* ssp.

6
20μ
7
8
10
9

TABLE IV*

	Chromosome															
	I		II		III		IV		V		VI		VII		VIII	
Genus *Pulsatilla*																
P. pratensis		.6–.2		.9–.3		1.1		2.2		2.9		3.3		3.8		3.9
		3.2		3.7		3.9		3.5		3.3		3.9		4.0		4.2
	5.3	4.0	4.1	4.9	3.5	5.0	1.6	5.7	1.1	6.2	1.2	7.2	1.1	7.8	1.1	8.1
P. nuttalliana ssp. *nuttalliana*		.6–.2		.8–.4		1.1		2.4		3.0		3.5		3.8		4.0
		3.1		3.4		3.8		3.8		3.5		3.9		4.1		4.3
	5.2	3.9	4.3	4.6	3.5	4.9	1.6	6.2	1.2	6.5	1.1	7.4	1.1	7.9	1.1	8.3
P. cernua (from Kurita)		.4–.2		1.0		1.0–.2		2.0		2.7		3.3		3.3		3.4
		3.2		3.2		3.5		3.0		2.7		3.4		3.7		3.8
	8.0	3.8	3.2	4.2	3.5	4.7	1.5	5.0	1.0	5.4	1.0	6.7	1.1	7.0	1.1	7.2
P. occidentalis		.8–.5		1.4		1.5		3.1		3.3		4.4		4.6		4.9
		4.6		4.7		4.9		4.4		4.1		4.8		5.2		5.2
	5.8	5.9	3.4	6.1	3.3	6.4	1.4	7.5	1.2	7.4	1.1	9.2	1.1	9.8	1.1	10.1

*See footnote, Table I.

Discussion

Basic chromosome numbers of 7 and 8 are found in the genus *Anemone* s. lat. Complements based on 8 chromosomes only were found in all species of the genus *Pulsatilla* studied, and in the section Eriocephalus of the genus *Anemone*. A basic number of 7 chromosomes only was found in species of *Hepatica*, and in the sections Anemonidium and Homalocarpus of the genus *Anemone*, while both basic numbers were found in sections Rivularidium and Anemonanthea. When the described arrangement is followed, namely with centromeres in a straight line and chromosomes generally in ascending order of size, certain basic patterns become apparent and in these, subgroups can be recognized. Admittedly chromosomes in comparable positions may differ in structure and yet be similar in gross appearance.

Examination of karyotypes based on 8 chromosomes shows that certain features are more or less general throughout. The long arms of the first three pairs are usually shorter than or do not exceed the long arms of the last pair in length. The karyotype of *A. quinquefolia* which forms an exception to this observation will be discussed later. The fourth pair with submedian constrictions may be equal to or shorter than the fifth pair in length but their long arms are somewhat longer to considerably longer than those of the fifth pair. The single known exception, where the fourth pair is unusually small, occurs in population 141 of *A. rivularis* which differs in this respect

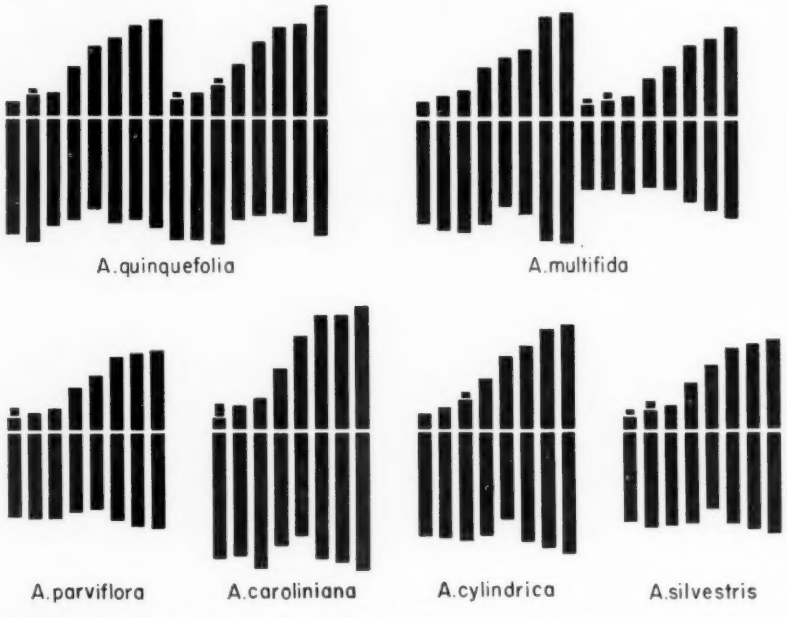

FIG. 11. Idiograms of species with basic complements of 8 chromosomes and arm ratios of 1:2 in Ch. IV. × ca. 3300.

from the other six populations studied in the series Rivularis. The basic pattern of the 8-chromosome series thus consists of three short-armed pairs with long arms not exceeding in length those of the last pair, a fourth pair with submedian constrictions, a fifth pair with the shortest of the long arms, and three remaining pairs of gradually increasing length, with median or near-median constrictions.

Karyotypes of the 8-chromosome series can be divided into two kinds, one in which the long arms of the fourth pair are twice as long or more than twice as long as the short arms, and another in which they are less than twice as long (1.4–1.8). The first ratio (1:2) is found in *A. parviflora*, the *A. virginiana–riparia–cylindrica* complex, *A. silvestris*, *A. caroliniana*, *A. quinquefolia*, *A. palmata*, and one chromosome set of *A. multifida*. The second

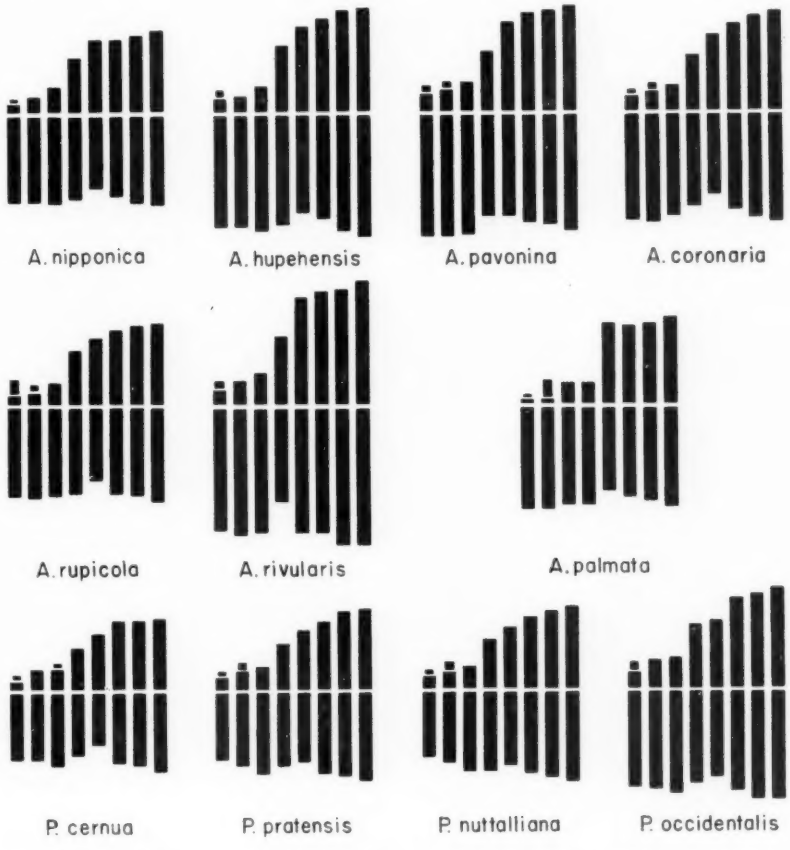

FIG. 12. Idiograms of species with basic complements of 8 chromosomes and arm ratios of 1:1.4–1.8 in Ch. IV and *A. palmata* with arm ratio 1:2+ in Ch. IV. × ca. 3300.

ratio (1:1.4–1.8) characterizes *A. pavonina*, *A. coronaria*, *A. hupehensis*, *A. nipponica*, *A. rupicola*, *A. rivularis*, all species of *Pulsatilla* which have been studied, and the second set of *A. multifida*.

As already mentioned, the karyotype of *A. quinquefolia* differs from others of the 8-chromosome series in respect to the longer arms of some of the short-armed pairs. Five of the six pairs of short-armed chromosomes have long arms exceeding those of the longest chromosomes in length. It may be noted that the karyotypes of *A. raddeana* var. *integra* and *A. pseudo-altaica* drawn by Kurita (18) indicate that, in these species, at least some of the pairs with subterminal constrictions resemble those of *A. quinquefolia* in this respect. The drawings also indicate that the fourth pairs have arm ratios of 1:2 as in *A. quinquefolia* rather than 1:1.6 as in *A. nipponica* with which they have been compared. If these observations are correct the karyotypes of these three species of the section Anemonanthea might constitute a third group of the 8-chromosome series.

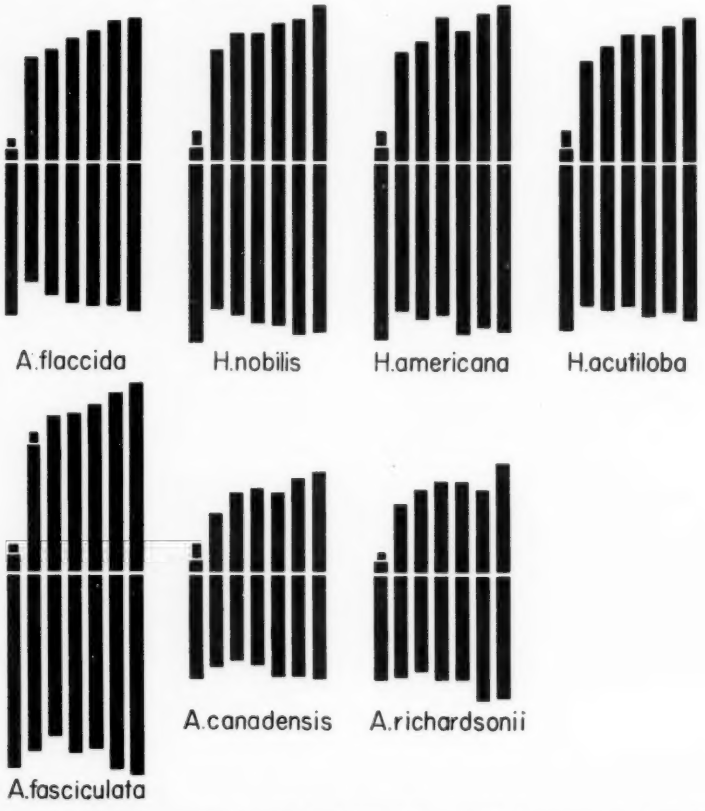

FIG. 13. Idiograms of species with basic complements of 7 chromosomes. × ca. 3300.

Since a pattern common to karyotypes of the 8-chromosome series can be recognized, those of the 7-chromosome series supposedly derived from them (26, 18) may be expected to reflect such a derivation. If the differences can be accounted for by a "Robertsonian fusion" (31) of two of the short-armed chromosomes of an 8-chromosome complement to give the additional long-armed chromosome of a 7-chromosome complement without visible alterations in other chromosomes, then the second chromosome pair of a karyotype of the 7-chromosome series will most likely correspond to the fourth pair of a karyotype of the 8-chromosome series.

Two main kinds of karyotype can be found in the 7-chromosome series, one which can and one which cannot be related to the patterns recognized in the 8-chromosome series. The first kind has the following characters in common with karyotypes based on 8 chromosomes: the long arms of the first pair are shorter than those of the longest pair; the long arms of the second pair are longer than those of the third pair, thus resembling the relationship existing between the fourth and fifth pairs of the 8-chromosome series; and the second pair have arm ratios of 1:1.4–1.6 which are frequently found in the fourth pair of the 8-chromosome series. This type is found in *A. richardsonii*, *A. canadensis*, and *A. fasciculata*. *A. narcissiflora*, related to the last taxonomically and cytologically, and although placed by Kurita (18) with *A. flaccida* of the next group, seems to belong here. The karyotypes of *A. richardsonii* and *A. canadensis* are remarkably similar to those known from 16-chromosome species in the absolute size of their chromosomes and in the arm ratios, about 1:1.6, of the second pairs. The similarity is less marked in the karyotype of *A. fasciculata* because of the large size of the chromosomes and the less common arm ratio (1:1.4) in the second pair.

The karyotypes of the second group are characterized by a combination of the following features: (*a*) the first pair of chromosomes have long arms exceeding in length those of any of the other pairs; (*b*) the second pair have the shortest of the long arms, and (*c*) arm ratios of 1:1.4 or less. In respect to the first point, this group resembles the karyotype of *A. quinquefolia*. Assuming that the second and fourth positions in the 7- and 8-chromosome series correspond, the second characteristic feature is not known in *Anemone* species outside the group being discussed, with the single exception of population 141 of *A. rivularis*. In species of other groups, the third or the fifth pair have the shortest long arms. Considering the last point, arm ratios of less than 1:1.4 are unknown and 1:1.4 infrequent in the second or fourth pairs of 7- or 8-chromosome basic complements of other species. Karyotypes of *A. flaccida*, *A. keiskeana*, *A. tagawae*, and species of *Hepatica* belong here.

In view of the general agreement between categories based on cytological results and those established on taxonomic grounds, discrepancies between the two classifications are worth noting. They occur principally within the sections Eriocephalus, Anemonanthea, and Rivularidium.

Within the section Eriocephalus, *A. silvestris* has been included in the series Anemonospermos where it is the only species with arm ratios of 1:2 in the

fourth chromosome pair instead of the 1:1.6 ratio of others in the group. *A. silvestris* crosses readily with taxa of the *A. virginiana–riparia–cylindrica* complex of the Virginiana series and with *A. multifida* of the Multifida series, with which it has arm ratios of the fourth pair in common (11, present investigations). It crosses with neither *A. hupehensis* nor *A. tomentosa* of the Anemonospermos series in which it has been placed (present investigations). No hybrids between species of the two contrasting kinds of karyotype (i.e. with 1:2 and 1:1.4–1.8 arm ratios in the fourth pairs) have thus far been obtained. The presence of both kinds of chromosome set in *A. multifida* indicates that such hybridization may have occurred in the past.

The section Anemonanthea is remarkable in the diversity of karyotypes in the species comprising it. Species of both the 7- and 8-chromosome series are present. Karyotypes of *A. flaccida*, subsection Stolonifera; of *A. keiskeana*, subsection Sylvia; and of *A. tagawae*, subsection unknown, all belong to the second group of the 7-chromosome series just described. Both kinds of 8-chromosome pattern are also found in this section. Karyotypes of *A. quinquefolia*, and possibly of *A. raddeana* var. *integra* and *A. pseudo-altaica*, belong to the first kind with arm ratios of 1:2 in the fourth pairs while *A. nikoensis* represents the second kind with ratios of 1:1.6 in its fourth pairs (18).

Kurita (18) proposed that all species of *Anemone* possessing karyotypes like that of *A. flaccida*, namely *A. keiskeana*, *A. tagawae*, and *A. flaccida* of the section Anemonanthea, should be included in the genus *Hepatica* or treated as a genus apart from both *Hepatica* and *Anemone*. There is some evidence for the desirability of separation of these species from the section Anemonanthea in the fact that their particular kind of karyotype cannot be related to either pattern of the 8-chromosome series known in this section or elsewhere in the genus. However, one feature in common with that of *A. quinquefolia* is present, as already pointed out, in the relationship between the long arms of the first and last pairs of the respective sets. Janchen (14) considered *Hepatica* to be more closely related to the section Anemonanthea than to other sections of the genus *Anemone*. On the basis of cytological evidence it would seem that this association should be primarily with taxa of Anemonanthea, of the 7-chromosome series. While Juzepczuk (16) established a subcategory to separate *A. flaccida* from *A. baicalensis*, $2n = 16$, and some related species of the subsection Stolonifera, he found no basis for more radical treatment.

Janchen (14) has reviewed the literature dealing with earlier proposals to separate the section Anemonanthea from the genus *Anemone*, and did not find these acceptable. The species concerned here all belong to the 8-chromosome series. Recommendations to segregate the section Anemonanthea or part of it from the genus *Anemone* have been based on cytological observations in the 7-chromosome series and on morphological studies in the 8-chromosome series. Decisions on the value of such segregation can be reached only after a complete investigation of the section.

The section Rivularidium contains species with 14- and 16-chromosome complements. The 14-chromosome karyotype of *A. richardsonii* has already

been discussed as being clearly related to the general pattern of the 8-chromosome series. This species extends from Greenland across the Arctic region of North America into northern Asia as far west as the Lena River. Sixteen-chromosome complements of the second group (arm ratio 1:1.4–1.8) have been found in taxa of the series Rivularis. The materials investigated in this series are native to the mountains of northern and southern India and to southeastern Asia. Thus a distinctive karyotype is found in species of each of these geographic regions.

Seven species of the section Rivularidium, all with restricted distribution, are found in South America (22, 23) and two, also with restricted distribution, occur in Mexico. *A. crassifolia*, which resembles *A. richardsonii* in having creeping rhizomes, is endemic to Tasmania. *A. tenuicaulis*, which Parkin and Sledge (27) describe as resembling *A. antucensis* of the high Andes of Chile and Argentine more closely than any of the species of the same section found in Asia, is restricted to northern New Zealand. This peculiar assemblage of widely separated endemic species has led to much speculation as to their origin and affinities. Cytological studies may contribute much to the understanding of this problem.

The present study has revealed an association of karyotypes having distinctive features with some categories at the species and higher levels in the genus *Anemone* s. lat. The results do not entirely support current classification and indicate the desirability of some changes, especially within the section Anemonanthea. Further investigation may be expected to contribute materially to a better understanding of the genus.

Acknowledgments

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AN ANATOMICAL STUDY OF THE SUBTERRANEAN ORGANS OF EUPHORBIA ESULA IN RELATION TO ITS CONTROL[1]

TRILOCHAN S. BAKSHI AND ROBERT T. COUPLAND

Abstract

Young roots of *Euphorbia esula* exhibit a structure similar to that of a typical dicotyledonous species. In older roots, wood comprises as much as two-thirds of the total tissues. Cork is several layers thick, and lenticels are of open type. Pericyclic meristem forms a continuous ring any part of which may give rise to lateral organs. The primordia of lateral roots and shoots cannot be distinguished morphologically from each other until they have emerged from the parent organ. In the case of stems the lateral buds originate endogenously from the internodes but exogenously at the nodes. The subterranean organs of the species are supplied with simple, nonarticulated, and branched laticifers. Almost all parenchyma cells including the laticifers are filled with starch grains. The significance of these anatomical features with respect to the control of the species is discussed.

Introduction

Throughout the life cycle of a plant there are changes in physiological balance, and each change brings about its own morphological and anatomical expression. Therefore, the successful control of persistent perennial weeds, like *Euphorbia esula* L., which propagate extensively by vegetative means, requires a thorough understanding of not only the external factors but also the conditions within the plant. It has been widely recognized (12, and several others) that the behavior of a plant is closely correlated with, among other things, its structure, and it is from the standpoint of structure that the problem of persistence of *E. esula* is considered here.

The vegetative propagation in *E. esula* is obtained by the development of innumerable buds on its underground organs. Coupland and his associates have studied not only the distribution of the subterranean organs and the vegetative buds on these organs (4, 5) but also the reproductive capacity of these buds (6). They found that the buds were most numerous on the uppermost portions of the subterranean organs, there being 42 buds per 15 cm length of a given organ, and that their number decreased to four for the same length at a depth of 105–120 cm. They further noted that 62% of the total buds on a given plant were present in the A horizon of soil. Although they obtained no evidence to indicate that shoots arising at depths greater than 43 cm in an undisturbed soil reach the surface, they were able to demonstrate that the shoots can emerge from the 90 cm depth after the upper part of the root system and soil was replaced by tamped, uninfested soil. This evidence seems to indicate that the subterranean organs of *E. esula*

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possess very active and efficient lateral meristems. The basic morphological problem in the vegetative propagation of this species, therefore, concerns a study of its lateral meristems, and the differentiation and emergence of primordia originating from these organs.

As far as the writers are aware, there are only two publications dealing with the detailed anatomy of the vegetative organs of *E. esula*. Hanson and Rudd (8) studied the structure of stem, root, and leaf. In stem they have described mostly the primary tissues, and in roots only the secondary tissues. Bakke (2) has presented a detailed account of the root and stem anatomy, and has given some excellent figures to illustrate it. Apparently no anatomical work has so far been reported on the origin and development of buds on the subterranean organs of the species.

Materials and Methods

The underground parts of *E. esula* were collected 6 miles southwest of Saskatoon, Sask. (SW. 34-35-6W3), on October 16, 1958, from a hole dug about two meters deep. Material was fixed in the field in formalin – acetic acid – alcohol, and organs from various depths were stored in separate containers for the purpose of comparisons. Since the roots of the species zig-zag their way through soil, minute soil particles adhere tenaciously in the concavities on root surfaces. Considerable care was required to clean these particles from roots without in any way injuring the organs. The usual methods of dehydration and infiltration were followed. Sections were cut 12 μ thick. Two combinations of stains, as described by Johansen (9), were used. Of these safranin and fast green gave better results than crystal violet and erythrosin. Best contrast in staining was obtained when the sections were immersed in safranin for 80 minutes, destained to the required intensity in picro-alcohol (approximately 8 seconds for woody and 4–5 for non-woody material), and counterstained with fast green for 5 seconds.

Observations

Root

In the younger roots, the usual three regions are visible: epidermis, cortex, and stele (Fig. 1). Epidermis consists of rectangular or oval cells with yellowish-brown cuticle on the outer walls. Cortex comprises 3–6 layers of cells of which the innermost, the endodermis, possesses Casparian strips. In some sections the inner and the radial walls of endodermis were observed to be somewhat thickened. In such cases Casparian strips were not visible. The remaining 2–5 layers of cortex consist of almost isodiametric parenchymatous cells. Stele is made up of the usual tissues of xylem, phloem, cambium, and pericycle. The arrangement of xylem varies from diarch to polyarch, triarch and tetrarch being the most common. Phloem occupies the angles between the primary xylem arms, and is separated from it by a single layer of cambium. The outermost layer of the stele is the pericycle. Starch is present mostly in the parenchymatous cells of the stele. The primary structure of root is thus similar to that of a typical dicotyledonous plant.

Secondary growth in the stele of *E. esula* is essentially similar to that reported for *Pyrus* (see 7). In older roots of *E. esula* with a diameter of 6 mm or more, wood forms nearly two-thirds of the entire section. Vessels are arranged in radiating rows, and tracheids and xylem sclerenchyma occupy most of the region between vessels. Vascular rays, both primary and secondary, are distinct, and are made up of parenchyma cells containing abundant starch. In wood, there are also present small groups of starch-bearing parenchyma cells. These groups are disrupted, more or less concentric, and are for the most part irregularly arranged. In this respect the present material resembles that of *E. cyparissias* studied by Korsmo (10). The unbroken concentric rings of "storage parenchyma" reported by Bakke (2) in *E. esula* were thus not observed in the material studied by the writers.

Shortly after the primary and secondary xylem form a circular and solid core in the center, the outer cells of the proliferated pericycle become meristematic, resulting in the formation of phellogen which produces cork towards the periphery and a few layers of phelloderm towards the center of root. From this stage most of the parenchyma cells of the root are filled with starch (Fig. 2). During the development of cork, therefore, the cortex and epidermis die and peel off. Korsmo (10) has reported a similar condition in *E. cyparissias*.

In roots which are 4–5 mm in diameter and which do not show any conspicuous annual rings, cork consists of 3–7 layers of suberized cells. Theoretically as more and more cells are added to the outside of the phellogen, the cork should become progressively thicker. However, in all the sections examined the writers found that this thickness does not exceed seven layers, the extra layers of cork being continuously peeled off the roots. In some cases the peelings become evident at the two-layered stage; however, they do not become separated from the parent organ until a few additional layers have been laid down.

In sections of older roots, numerous lenticels in various stages of development are visible. They do not seem to be distributed in any definite relation to the inner tissues of the root. At irregular intervals along the circumference of the phellogen, there are produced complementary cells which push their way through the outer corky layers. The smooth root surface is finally ruptured, and an open type of lenticel without any recurved margins is formed.

Bakke (2) has stated that after secondary growth "the entire root" of the species "is alive." That this is not the case is readily seen from the fact that nearly two-thirds of the root consists of dead tissues, viz. vessels, tracheids, xylem sclerenchyma, and cork.

At about the stage when the differentiation of secondary xylem has set in, cells of pericycle opposite the protoxylem points become meristematic. The meristematic activity gradually spreads to the remainder of the pericycle resulting in a continuous ring of pericyclic cambium (Fig. 3). The primordia of lateral organs may arise from any part of this pericyclic meristem (Figs. 4 and 5).

Tissue differentiation within a lateral organ primordium has been observed to commence at about the stage when its tip lies next to the outer layer of cortex. Even at this stage the differentiation has not advanced far enough to indicate whether the primordium is that of a root or a shoot. Nearly two hundred slides, each with 25–200 sections (the number varying with the size of organ cut), were examined. In every case a shoot primordium could be identified morphologically only after it had emerged from the parent root (Fig. 5).

Coupland and his associates (4, 5, 6) carried out a detailed study of the distribution and abundance of vegetative buds on the underground organs of *E. esula*. The validity of their conclusions could have been questioned to a certain degree since their observations were based on only such buds as had emerged from the subterranean organs; however, since the buds can be recognized as such only after their emergence from the parent organ, this study conclusively supports the method for bud identification used by these authors.

It is interesting to note that roots of about the same size but collected from different depths showed similar anatomical features.

Stem

The primary and secondary structure of stem has been adequately described by Bakke (2). In the material studied by the writers, stems and roots with about the same diameter had approximately the same amount of vascular tissue. Bakke (2), however, records that "unlike the roots, the xylem of the stem has comparatively little vascular tissue." A critical examination of his Fig. 22 revealed that even in his material the vascular tissue in general and xylem in particular were in about the same proportion in comparison with the entire stem as in the writers' material. The discrepancy in Bakke's observations seems to have arisen because he considered tracheids as "sclerenchymatous tissue" which, according to him, forms "the largest portion of the stem." Actually, more than three-fourths of this "sclerenchymatous tissue" consists of tracheids which, together with vessel members, are, according to Esau (7), "tracheary elements."

The lateral organs on the internodal regions of underground stems originate endogenously. However, at the nodes, exogenous origin appears to be the rule.

Root-to-stem transition has been observed in the region 15–22.5 cm below the soil surface. Hanson and Rudd (8) have stated that the stem "may

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FIG. 1. Transection of young root. The lateral root, cut longitudinally, has originated from the pericycle opposite the protoxylem. Note the accumulation of most starch in stelar parenchyma. ×100

FIG. 2. Transection of older root showing accumulation of starch in parenchyma. Cells of cortex and epidermis are dead, and are about to be peeled off. ×50

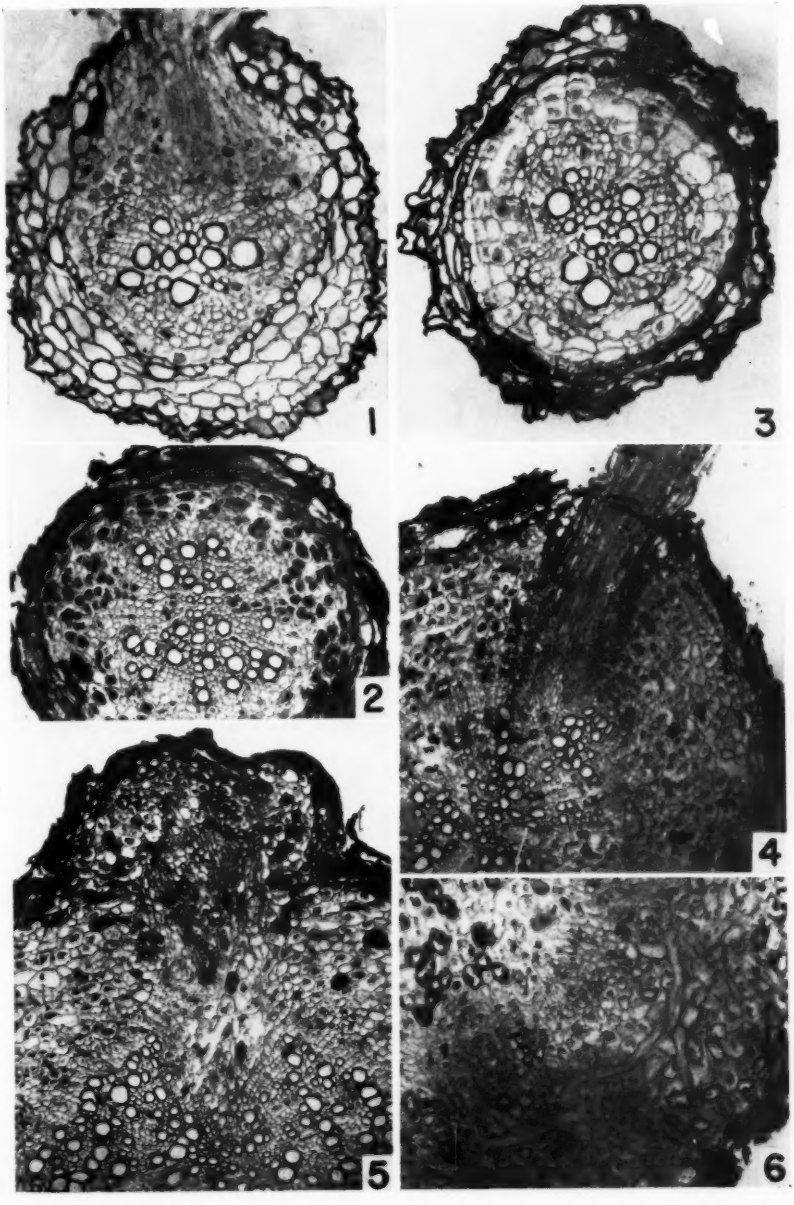
FIG. 3. Transection of root showing an unbroken ring of pericyclic cambium. ×85

FIG. 4. Transection of root. Lateral root has originated from pericycle between the protoxylem arms. ×50

FIG. 5. Transection of root showing lateral bud which originated endogenously opposite the protoxylem. ×50

FIG. 6. Transection of young stem showing laticifers. ×150

PLATE I



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extend underground a foot (30 cm.) or more before a transition region is reached." It is possible that they were studying stems which had originated as lateral buds on the main root at that depth, and which had outgrown the original stem that had developed from the plumule. This is to be expected, particularly in cases where the original stem is severed due to disturbances in the top soil. The occurrence of such a possibility is further confirmed by the observation of Coupland *et al.* (6) that severence of roots at various depths increased the number of buds below the cut.

Laticiferous Tissue

All subterranean organs of *E. esula* are profusely supplied with laticiferous tissue. The laticifers are simple, nonarticulated, and branched. In the younger organs most of the laticifers run along their lengths. On the other hand in the older roots and stems where the secondary cortex is many-layered, the laticifers are cut at almost all angles (Fig. 6). The present study confirms the findings of Hanson and Rudd (8) that latex of *E. esula* contains "cigar-shaped starch grains."

Discussion

Anatomical features which appear to be significant with respect to the persistence of *E. esula* include the formation of several-layered cork, the accumulation of starch in various tissues of subterranean organs, the development of a continuous ring of pericyclic meristem in roots, and the characteristic growth of lateral organs from this meristem.

Cork

It has been seen that in most roots producing lateral organs there are usually 3–7 layers of cork cells. Cork thus forms protective covering for roots. As noted earlier, the lenticels are of the open type. According to Swingle (14), Lugovoy (11) studied the correlation between type of lenticel development and ability to root as stem cuttings of a number of tree and shrub species. He concluded that species characterized by a particular open type of lenticel were readily propagated by stem cuttings. Because the writers have neither studied the lenticel development on the stem of *E. esula* nor correlated the lenticel type found on its roots with the ability of this species to propagate extensively by buds on these organs, and since comparable information on other persistent weeds is wanting, a definite conclusion in this respect cannot be reached. The possible existence, however, of such a correlation should not be overlooked in future investigations.

Accumulation of Starch

Hanson and Rudd (8) found that cells of medullary rays and cortex of *E. esula* roots contained starch. Starch was also seen by them in the latex. Extensive accumulation of starch in most of the parenchymatous tissue of roots has been observed by Bakke (2). He has described the presence in older roots of alternating rings of xylem and "storage parenchyma", and has concluded that since the food storage is carried out by both the cortex and

the rings of "storage parenchyma" the plant is able "to survive long periods of shading or clipping and still have stored food available to start new and vigorous growth." In the material studied by the writers distinct "storage parenchyma rings" were not present. However, almost all parenchyma cells showed the presence of starch. Starch was observed in some of the cortical cells as early as the stage when the first secondary xylem cells have been laid down. When phellogen is developed most of the parenchyma cells in the stele contained starch. Latex of the plant was also full of starch grains. In all cases the presence of starch could be easily detected by the iodine test.

According to Yerkes, Scott, and Swingle (16) success or failure as understocks in "commercial rose growing" was dependent upon the stage of maturity of the stock at the time of digging, and that many cases of unsatisfactory results reported with apparently well-grown "manetti stocks were really due to digging in an immature condition." They found that a simple iodine test on the older stems could be used as a safe criterion for digging, abundant starch signifying satisfactory maturity. Although the total amount of organic reserves in the underground organs of *E. esula* varies from month to month during April–November, they are never completely depleted of these reserves (1). Hanson and Rudd (8) noted that in late fall and in early spring the parenchymatous cells "were filled with starch." The writers found that even the youngest roots examined contained starch grains, and that the vegetative buds on roots are not produced until starch had accumulated in most of the root parenchyma cells. Could it be then that the ability of this species to propagate vegetatively by means of subterranean organs is directly correlated with the extensive starch accumulation in these organs? Only further research along these lines could furnish a satisfactory answer.

In young roots with little or no secondary xylem, most of the starch is accumulated in the stelar parenchyma. Since phellogen originates in the pericycle, the epidermis and cortex are peeled off due to the formation of cork. The accumulation of starch mainly in the stele of younger roots may, therefore, be of adaptive significance, particularly in view of the fact that shoots have been observed to develop on roots after the appearance of cork.

Lateral Meristem and Lateral Organs

The most significant finding of the present study is the presence of a continuous ring of pericyclic cambium in *E. esula*. Owing to the presence of such a ring, the distribution of lateral organs on roots of the species does not follow the laws established by Van Tieghem (15). While in younger roots lateral organs may originate from any part of the pericyclic cambial ring, on older roots lateral organs arise generally from the pericycle opposite the protoxylem points, although in several cases they have been seen to originate from any part of the pericycle between the protoxylem arms. During the present investigation a total of 48 lateral organ primordia in various stages of development were examined. Of these, 14 were found to have originated from the pericycle lying between the protoxylem points. The pericyclic

cambial ring thus adds considerably to the potentiality of this species in the production of lateral organs.

Once the initials of lateral organs are formed, they continue to divide and redivide resulting in groups of meristematic cells that form the primordia of lateral organs. The primordia, as evidenced by the crushed or distorted shape of cortical cells of the parent root, push their way through the cortex. The present study, therefore, does not support the conclusion of Smith (13) that the lateral organ primordium dissolves its way through the cortical cells of the parent root, rather it seems to corroborate the observation of Priestley and Swingle (12) that "the emergence of root primordium is from actual rupture of the mother tissue."

This study has revealed that tissue differentiation in primordia of lateral organs developing on roots does not take place while the primordia are within the parent organ. In its earlier stages of development, therefore, a shoot primordium cannot be distinguished morphologically from a root primordium. This appears to suggest that the primordia, until their emergence from the mother root, are potentially capable of forming either a root or a stem. That this is not improbable is suggested by the observation of Beijerinck (3), who described the transformation of a typical shoot apex into a root meristem. If, then, each lateral organ primordium on the roots of *E. esula* is potentially both a root and a stem, its ultimate fate being determined probably by the external environment, the potentiality of this species to propagate vegetatively, in view of what has been stated earlier with respect to its lenticels, starch accumulation, and pericyclic cambial ring, attains alarming proportions.

Acknowledgments

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GENERATIVE CYCLES OF PROTEIN AND TRANSAMINASE IN THE GROWING CORN RADICLE[1]

F. S. Cook[2]

Abstract

Measurements have been made of growth rates, cell numbers, fresh and dry weights, protein and soluble nitrogen levels, and glutamic–aspartic transaminase activity in six successive 2 mm segments of the radicles of 3-day-old corn seedlings. The measured quantities of protein and enzyme activity are related to the stage of average cellular development, to a linear distance scale along the axis of the radicle, and to the time scale. Increments per cell per hour during cell growth are therefore computed. An attempt is made to explain the significance of the genesis of the transaminase in the growth and development of the radicle cells, to the concurrent genesis of total and specific protein, and to other generative cycles.

Introduction

The reactions catalyzed by the transaminases have received considerable study, but the significance of these reactions in the metabolism of the living cell and the intact organism is still not fully clear. It has been suggested that they interconvert amino acids in such a way that the proper balance is maintained for the synthesis of the specific proteins in every type of tissue or cell (2, 19). Studies aimed at disclosing the degree to which transaminase activity is associated with active protein metabolism are inconclusive. Cohen and Hekhuis (20) found a low rate of transamination in mouse tumors, fetal cats, and regenerating rat livers in which protein synthesis was rapid; and Smith and Williams (41) found no close correlation between the enzymatic activity and protein synthesis in the seedlings of several higher plants. Albaum and Cohen (2), on the other hand, propose a close relationship based on experiments with whole oat seedlings at various stages of germination. It was found that a sudden increase in the rate of synthesis of protein per seedling, beginning between 72 and 96 hours from germination, was preceded by about 24 hours by an acceleration in the increase of transaminase activity and soluble nitrogen per seedling. Protein synthesis, they claim, is accelerated only after the concentration of amino acids and the transamination activity have reached a certain level.

It is known that in a germinating seed non-specific, storage proteins are hydrolyzed, and amino acids and amides translocated to the growing regions of the radicle and shoot. In these regions new proteins, including and probably consisting chiefly of specific proteins, are synthesized, and amino acids in different proportions and new amino acids will be required (5, 16). Yemm

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(51) has shown that in barley seedlings 90% of the nitrogen required for the synthesis of amino acids is derived from amides, glutamic acid, and proline of the reserve proteins. Panalaks (34), in a study of nitrogen metabolism in soybean seedlings, found that large amounts of glutamic acid were released from the proteins in the cotyledons, and a balance of nitrogen fractions suggested to him that this glutamic acid is converted to asparagine. During the 20- to 25-day germination period when transaminase activity per unit weight of fresh tissue was near its maximum value in the cotyledons, the loss of glutamic acid could account for all of the asparagine found in the seedling axis. Panalaks suggests that glutamic acid is converted to aspartic acid by transamination followed by amidation to give asparagine. Given a supply of the correct keto acids, it is conceivable that the transaminases could carry out many such necessary transformations.

The suitability of the radicle of higher plant seedlings for the study of growth and associated phenomena has been recognized since the marking experiments of Sachs (40) and the histochemical experiments of deVries (46, 47, 48) in the last century. The most extensive work has been that of Brown and his students (6, 7, 8, 9, 10, 11, 12, 13, 37, 38, 50). They have determined rates of cell division and cell elongation, and made measurements of dry weight, protein nitrogen, respiration, and the activities of several enzymes on the radicles of peas and broad beans. Other studies have been made on the apex of the broad bean radicle: Morgan and Reith (33) have investigated the amino acid composition and quantitative levels of free amino acid, protein, and peptides; Robinson (36) has assayed for proteolytic enzyme activity; and Robinson and Cartwright (39) have measured the activities of nucleolytic enzymes.

Erickson and Goddard (25) developed an elegant method for measuring growth rates of corn radicles by marking them with lamp black particles and making a continuous photograph of the growing radicle on a moving film. From the slopes of curves so obtained they could compute the rate at which any point on the root was elongating—the relative elemental rate of elongation. They measured, as well, cell size, cell numbers, fresh and dry weights, and total and protein nitrogen, and calculated the elemental rates of change of these values.

Baldovinos (3) obtained similar data on corn, but did not determine relative elemental rates of change. Jensen (27, 28) has made a careful study of the morphology, cell size, cell number, total carbohydrate, protein and soluble nitrogen, nucleic acid, and oxygen uptake of the first 3 mm of the radicle of the broad bean and the onion.

The purpose in view of the studies reported upon here is to relate the genesis of glutamic–aspartic transaminase to the growth and development of corn radicle cells, to the concurrent genesis of total protein and (to a rough, first approximation) of specific protein, and to other generative cycles. It is possible to relate the measured quantity of protein or enzyme to the stage of average cellular development, to a linear distance scale along the axis of the

radicle, and to the time scale. It has been possible therefore to compute increments per cell per hour during cell growth.

Some of the properties and the kinetic behavior of the glutamic–aspartic transaminase of corn radicles have been described previously (21, 22, 23).

Materials and Methods

Seedling Material

Segments of the radicles of golden Rocket hybrid corn were used in all experiments. The seeds were selected, treated, and germinated as described in a previous paper (21). The segments were excised by hand with a razor blade on one half of a 15-cm Petri dish placed over a sheet of ruled paper, and quickly transferred with forceps onto dampened filter paper placed in a closed Petri dish. Instruments, Petri dishes, filter paper, and water were all sterile. While awaiting further treatment the radicle tips were kept on the filter paper in the refrigerator for a period never in excess of 2 hours.

Enzyme Preparations and Reactions

In most experiments described here fast frozen radicle segments were used as the source of transaminase activity. These were simply frozen immediately after excision either on a block of dry ice or in a deep freeze and placed, in the frozen state, into the substrate–buffer solution. The latter consisted of 3.0 ml of 0.2 *M* phosphate buffer of pH 8.0, 1.0 ml of 0.1 *M* L-aspartic acid, and 1.0 ml of 0.1 *M* α-ketoglutaric acid.

In a few cases the segments were homogenized in the cold phosphate buffer in a glass homogenizer. The tube of the homogenizer was kept immersed in ice water during this operation. The structural parts of the tissue were tossed down by a 5-minute centrifugation at 2000 r.p.m. The cloudy, yellow-brown supernatant was dialyzed for 12 hours against phosphate buffer in the refrigerator, and used without further treatment. The reaction mixture was the same as that described for frozen segments except that 4 ml of the homogenate replaced the segments and the buffer. Details of the comparison between these two enzyme sources have been published (21).

L-Aspartic acid and α-ketoglutaric acid were purchased from Nutritional Biochemicals Corporation, and their solutions were adjusted to pH 8.0 with potassium hydroxide before use.

The transamination reaction was carried out with shaking and stopped after a 10-minute period with six drops of 6 *M* orthophosphoric acid, and 0.5 ml of 10% sodium tungstate. This precipitates the protein.

Analytical Procedure

Where intact segments are used these are filtered off and washed. The precipitated protein is centrifuged down, and the supernatant transferred with washings to a tube, and placed in a boiling water bath for 2 hours. This reduces the volume, and destroys keto acids. The solution is then allowed to cool, made up to 10 ml, and analyzed for its yield of carbon dioxide in the ninhydrin reaction (44). The difference between the carbon dioxide yield

at the end of a period of transamination and the yield from a system in which the reaction was stopped at zero time is a measure of the extent to which glutamic–aspartic transamination has proceeded. Details of this method have been described elsewhere (21).

Nitrogen Determinations

All nitrogen determinations were made by an adaptation of the micro-Kjeldahl technique of Ma and Zuazaga (31). Total nitrogen was determined by simply digesting a suitable number of dried radicle segments. Partitioning of soluble and protein nitrogen was accomplished by an alcohol precipitation method developed by Duff and co-workers (22, 24). This method measures both soluble and insoluble nitrogen directly, and allows one to compare the sum of these two values with the value for total nitrogen determined on whole segments. Discrepancies may be as high as 10% in the apical region; in more mature parts of the radicle they are considerably less. Such losses are largely accounted for in the centrifuged cell wall material.

Cell Counts

The cells of corn radicles are difficult to separate from one another by chemical or enzymic techniques and several unsuccessful attempts were made during these studies. The procedure finally adopted was to let the segments sit for 5 days at 30° C in a 10% pectinase solution (Nutritional Biochemicals) protected with a few drops of toluol. The pectinase was dissolved in citric acid (0.1 *M*) – potassium monohydrogen phosphate (0.2 *M*) buffer of pH 3.6. During the incubation period the suspensions were frequently shaken and stirred with a small glass stirring rod. Twenty segments were used for each count, and each suspension was diluted to 0.5 ml with water prior to reading. Counts were made on an improved Neubauer haemacytometer.

Results

Sampling and Cell Heterogeneity

In an effort to shed some light on the relation between the genesis of transaminase, protein synthesis, and growth a study was made of glutamic–aspartic transaminase activity, protein levels, and cell numbers in six successive 2 mm segments of the radicle of 3-day-old corn seedlings (Figs. 1–4). In one experiment the procedure was varied somewhat in an effort to obtain meristematic tissue, elongating tissue, and differentiated tissue separate from each other and as homogeneous as possible. The first 2 mm segment was excised and used, the next 0.5 mm piece was discarded, and the succeeding 2.5 mm segment was cut off and used. The entire segment from a point 5 mm from the tip to 15 mm from the tip was discarded, and the 5 mm segment proximal to this was studied (Fig. 4B). The segments studied in terms of distances from the tip of the radicle are as follows:

0–2 mm, mitotic cells;
2.5–5 mm, elongating cells;
15–20 mm, cells neither dividing nor enlarging.

Complete homogeneity of cell type is, of course, unattainable even in a root. There are obvious morphological differences in cells from even a very small segment; this has been emphasized by Jensen (27), and Brumfield (14). The growth regions that the botanist has defined are not sharply separated from one another, and groups of specialized cells occur in all regions. Clowes (17, 18), for example, has shown that in corn roots there exists a small part of the meristem just proximal to the base of the root cap in which the cells rarely divide or grow. They fail to synthesize DNA from externally supplied phosphate and adenine, and when compared with surrounding cells, their rate of incorporation of leucine into protein is low, and they have smaller nucleoli, and lower content of cytoplasmic RNA. In the broad bean Jensen (27) has separated the distal 3 mm into six zones, on both morphological and chemical grounds. Nevertheless, segments 2 mm or less in length and critically selected for position along the radicle axis undoubtedly contain a high percentage of cells which are much alike with respect to their ontogenetic development. The work of many students of root tip growth and metabolism has clearly indicated that physiological and biochemical data obtained on such segments can be correlated with the ontogenetic state of most of the cells in a particular segment with considerable accuracy.

Work in this laboratory (24, 35) has shown that another source of heterogeneity in a sample of root tips is variation among individual roots. It is apparent upon examination of the 30 seeds within one Petri dish that there is variation in the time at which the radicle bursts from the coleorhiza, and in the subsequent tempo of growth. As growth proceeds the diameter of the terminal centimeter diminishes, and the mass of this segment is less on the third day from germination than it is 50 hours from germination. The dry weight also changes in a similar manner. Respiration rates fall slightly from a maximum initial value as growth proceeds, and Q_{O_2} values vary considerably because of changes in both respiratory rate and dry weight. Furthermore, it has been shown that the Q_{O_2} value of the 1 cm apical segment is higher when it has been produced by a large seed than by a small seed even when the radicles are of equal length (35).

In order to reduce such variability the seeds were individually selected by hand and eye. Chiefly small seeds, but also excessively large ones, were discarded. The time of excision was always within 68 to 72 hours from the time of setting out on agar, and the radicles selected were from 6 to 10 cm in length. Duplicate experiments were found to agree closely, and each datum presented in this study is the mean of at least two determinations. Figure 4B presents data from two separate experiments on protein nitrogen levels per cell and the agreement is seen to be good.

Cell Numbers

Cell numbers per millimeter are plotted in Fig. 1. These data were obtained primarily in order to be able to compute nitrogen fractions and enzyme activity on a cellular basis, but they also reveal information on cell enlargement. It is evident that maximum cell enlargement occurs between the

first and second segment, and that some enlargement goes on up to 8 mm. Erickson and Goddard (25) find in their corn radicles that the maximum elemental rate of elongation (extension growth) occurs between 3 and 4 mm from the tip, and the maximum elemental rate of change in cell numbers (mitotic growth) is at 1.5 mm from the tip. The absolute values for cell numbers per segment measured for corn in these studies, for corn by Erickson and Goddard, and for pea by Brown and Broadbent (8) are all of the same order.

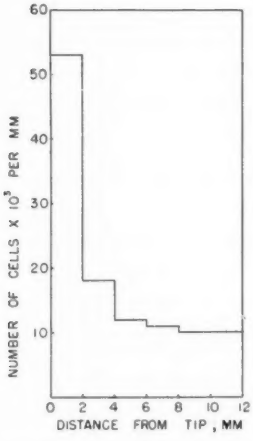

FIG. 1. Haemacytometer counts of cell numbers per unit length of corn radicle.

Brown and Broadbent, and Erickson and Goddard, used smaller sections than were employed in these studies, and therefore, Fig. 1 fails to show the initial rise from the low value at the root tip to the maximum at 1.0 to 1.5 mm from the tip found by these students. This applies to many of the other data, as well. Brown and his students studied 1 mm segments, and therefore, the center of their apical segment is 0.5 mm nearer the radicle tip than the center of the apical segment employed in the studies recorded here. This must be taken into account when comparing the two sets of data with respect to the increments of enzyme activity and nitrogen levels from the mitotic segment to the segment of full elongation.

Fresh and Dry Weights

Fresh weights per millimeter and fresh weights per cell are shown in Fig. 2. These histograms agree closely with the corresponding curves of Erickson and Goddard. Both sets of data of fresh weight per section show a levelling off in the initial rate of increase around the 3 mm mark, and then a further small increase. In Fig. 2A fresh weight levels off around 5 mm, but Erickson and Goddard found a more gradual increase from the level portion of their curve up to 10 mm.

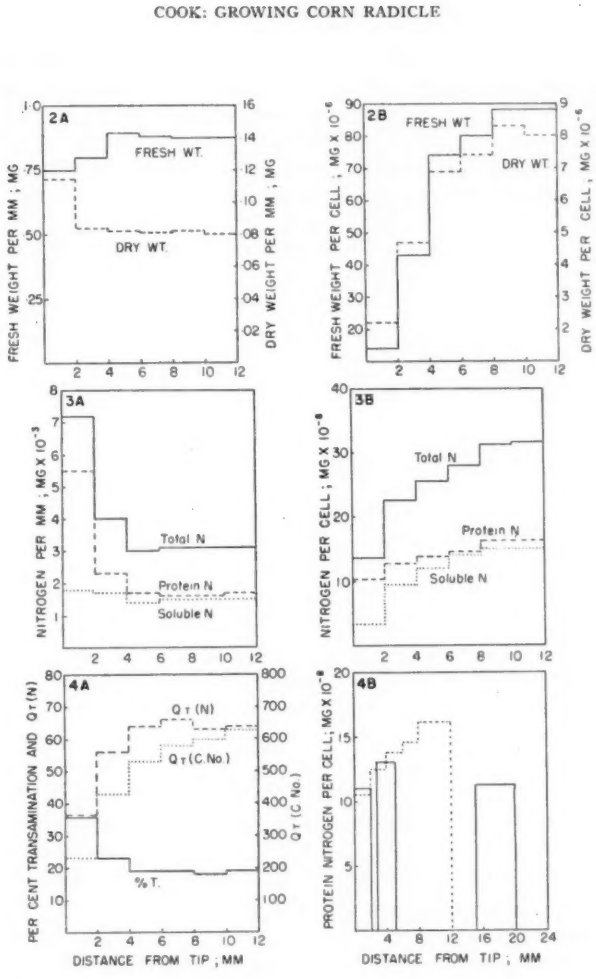

FIG. 2A. Fresh and dry weights per unit length of corn radicle. FIG. 2B. Fresh and dry weights per corn radicle cell.

FIG. 3A. Average content of total nitrogen, protein nitrogen, and soluble nitrogen per unit length of corn radicle. FIG. 3B. Average content of total nitrogen, protein nitrogen, and soluble nitrogen per corn radicle cell.

FIG. 4A. Per cent transamination per unit length of corn radicle, % T; per unit of protein nitrogen per hour, Q_T(N); and per cell per hour, Q_T(C.No.). Glutamic–aspartic transaminase reaction *b*.

System and conditions of reactions : 100 frozen radicle segments, 2.0 mm in length; 3.0 ml phosphate buffer, 0.2 *M*, pH 8.0; 1.0 ml of 0.1 *M* L–aspartic acid; 1.0 ml of 0.1 *M* α-ketoglutaric acid. Temp. 38° C. Reaction time, 10 minutes.

FIG. 4B. Average content of protein nitrogen per corn radicle cell. The histogram for protein nitrogen per cell from Fig. 3B is superimposed in dotted lines.

The meristematic cells have a comparatively low fresh weight (Fig. 2B). Maximum fresh weight per cell is attained between 8 and 10 mm which corresponds to the point at which elongation ceases (25), and cell numbers per section reach their minimum (Fig. 1). Maximum elemental rate of increase in fresh weight occurs between 3 and 5 mm from the tip (25).

Dry weight per millimeter and dry weight per cell are also shown in Fig. 2. Erickson and Goddard, and Brown and Broadbent, show a rapid increase from zero to about 2 mm. Figure 2A shows a corresponding high value for the entire 2 mm apical segment, followed by a rapid drop to a constant lower value proximal to the 4 mm point. Dry weight per cell decreases during the first millimeter in both corn and pea (8, 25), and then rapidly increases (Fig. 2B). It will be seen that there is a slight levelling off in the rate of increase between 4 and 5 mm. This feature is found in the data of Erickson and Goddard as well.

Total and Protein Nitrogen

Total nitrogen per millimeter (Fig. 3A) is high in the protoplasm-packed mitotic cells, and decreases reaching a constant minimum value about 5 mm from the tip. Total nitrogen per cell (Fig. 3B), on the other hand, is low near the tip and increases to a constant maximum value between 8 and 10 mm.

Protein nitrogen per millimeter gives a curve much like that for total nitrogen (Fig. 3A), and protein nitrogen per cell increases from the tip to a maximum between 8 and 10 mm from the tip (Fig. 3B). These data differ in some respects from the results of other students. Erickson and Goddard found an increase in protein nitrogen as a cell was displaced from the tip to a rather broad maximum at the 5 mm region. From 5 to 8 mm, however, there was a marked decline to a constant value only slightly higher than that of most of the mitotic cells. The results of Brown and Broadbent (8) with pea are very similar, although their 5 mm maximum is sharper. Baldovinos (3) reported that in corn radicles both total and protein nitrogen decrease between the 3rd and 5th mm segments. Robinson and Brown (37) found a sharp drop from the maximum in broad bean roots, but the maximum was not reached until the 8 to 10 mm region.

Figure 3B shows no evidence of any protein loss, but Fig. 4B shows clearly that there has been a sharp drop between the 10–12 mm section and the 15–20 mm section. The protein nitrogen histogram from Fig. 3B has been superimposed (dotted lines) upon Fig. 4B, and it is evident that the two sets of experiments are comparable, and that differences between the present data and those referred to above lie only in the points at which protein increase per cell ceases and decline begins. These differences are probably referable to species and varietal variation in the materials, and to differences in the age of the seedlings.

Jensen (27) has claimed that in the broad bean cells lose protein as they elongate. The discrepancy between his results and those of most other workers are attributable to his cell counts.

Soluble Nitrogen

Histograms showing total soluble nitrogen per millimeter and soluble nitrogen per cell are shown in Fig. 3. There is not a great variation from segment to segment, the most distal two segments having only a slightly higher content than the others. On a cell basis, however, soluble nitrogen increases sharply from the apical 2 mm to a constant maximum value at the 8–10 mm region.

Morgan and Reith (33) found a tenfold increase in free α-amino nitrogen and a twentyfold increase in peptides between the 1.4–2.6 mm segment and the 7.6–8.8 mm segment of broad bean roots. Using paper chromatography these students showed a variation in amino acids from section to section. All amino acid containing fractions from the meristematic zone yielded a variety of amino acids which were not found in other regions.

Transaminase Activity

Glutamic–aspartic transaminase activities in the six 2 mm segments of the corn radicle are shown in Fig. 4A, expressed in three different ways. Determinations were made by the ninhydrin method using 60 fast-frozen intact segments (21). A second series of experiments was conducted using only three selected segments from the three growth regions as described in the section on sampling and cell heterogeneity. In this case activity was again measured by the ninhydrin method, but an homogenate was used as the enzyme source, and therefore, Q_T(N) values were higher. The two experiments can be shown to be comparable, however, since they involve common segments of the apical 5 mm, and changes in activity based on both protein nitrogen and cell numbers are of the same order in both.

$$Q_T(\mathrm{N}) = \frac{\mu\mathrm{M\ aspartic\ acid\ transaminated}}{\mathrm{mg\ protein\ N} \times \mathrm{hours}}.$$

This expression was proposed by Leonard and Burris (29). Protein nitrogen is replaced by cell number to give Q_T(C.No.) values.

Figure 4A shows that per cent transamination is highest in the apical segment, and has reached a constant minimum value, which is only about 50% of that of the 0–2 mm region, by the 4–6 mm segment. Q_T(N) values, however, are low in the apical segment and increase rapidly to the 6–8 mm segment. The differences in values between the last four segments are small and probably not significant. The maximum value is nearly reached by the 4–6 mm segment. There is a suggestion of a decrease in activity proximal to the 6–8 mm zone, but if real it is small. However, a measurement of activity with an homogenate of segments of the 15–20 mm region shows that there has been a considerable fall in activity by the 15–20 mm region. As cells are elongating protein increases, and the proportion of this protein specific for transamination also increases.

This is shown also by comparing the Q_T(C.No.) values of Fig. 4A with changes in protein per cell. While the latter is increasing 1.5 times from the 0–2 mm segment to the 8–10 mm segment, transaminase activity per cell

is increasing more than 2.5 times. Values for Q_T(C.No.) increase up to at least the 10–12 mm segment, but again it has been shown there is a slight decline in activity by the 15–20 mm segment.

The most extensive data on enzyme activity in root tips have been obtained by Robinson and Brown (11, 36, 37, 38) and by Robinson and Cartwright (39) using broad bean. All enzymes studied except dipeptidase, ribonuclease, and desoxyribonuclease showed a decrease on a per cell basis from near the tip back to near the 2 mm point. All then showed a sharp increase in activity, phosphatase, dipeptidase, and invertase having a maximum close to the 8 mm point, glycine oxidase nearer 9 mm, the proteolytic enzymes at 10 mm, and the nucleolytic enzymes near 12 mm. An equally abrupt decline in activity followed the maximum, except in the case of the nucleolytic enzymes.

Discussion

Radicle cells in stages of development from mitotic to fully enlarged and vacuolated are included in the observations reported in this study. In further considering the data it is convenient to begin with the cells in the apical segment.

The Mitotic Stage

The apical 2 mm of the corn radicle contains practically all of the cells in mitosis. An examination of 16 longitudinal sections of corn root tips revealed no mitotic figures beyond 2.1 mm from the tip. Erickson and Goddard (25) found none beyond the 2.5 mm mark.

The cells are non-vacuolated and filled with dense protoplasm; water content per unit mass is relatively low, but dry matter on the same basis is high. Calculated on a unit mass basis, total nitrogen, nitrogen fractions, enzyme activity (11), and rate of respiration (26, 32) are high. Several workers (4, 8, 49), using smaller segments, have shown that the maximum Q_{O_2} value is actually reached slightly behind the apex, but well within the 2 mm region.

Such observations as these suggest high rates of metabolism, but when the various metabolic activities are expressed in terms of cells, or unit of protein, it is seen that the relative rates are comparatively low. Total nitrogen and protein nitrogen per cell is low, and respiration (8, 24), and enzymatic activity (37) per cell, or per unit protein nitrogen are low. Apparently much of the protein in these cells is functionally non-specific and relatively inert at least with respect to the metabolic activities discussed here. Some protein synthesis is probably occurring since new cells are being formed, and there is a certain amount of enlargement as the cell traverses the meristematic region (8, 14, 25). Both transaminase and proteinase (11) show some activity per cell in this segment, and may be associated with a slight protein turnover. Compared with their maximum values in the radicles transaminase would appear to be the more active enzyme in the apical region.

The Enlargement Stage

Although cells are enlarging in the meristematic zone to some extent, the region from 2 mm from the tip to 8 or 9 mm from the tip is one which encompasses most of the active extension growth in the corn root. The cells are vacuolating by taking up quantities of water, and their fresh and dry weights, soluble and protein nitrogen levels, and transaminase activity are all increasing. Table I shows the coefficients of increase of these cellular properties during the growth of a cell from the 0–2 mm segment to the 8–10 mm segment, where elongation is complete. Respiration per cell also increases rapidly (8).

TABLE I

Coefficients of increase of fresh and dry weights, nitrogen fractions, and transaminase activity during cell elongation in the corn radicle

	Coefficients of increase per cell*
Fresh weight	6.3
Dry weight	3.7
Total nitrogen	2.3
Protein nitrogen	1.5
Soluble nitrogen	4.5
Transaminase activity†	2.6

*Based on a comparison of the segment 8–10 mm from the tip with the apical 2 mm.
†Glutamic-aspartic transaminase, reation *b*.

The elongated cell has increased its fresh weight by more than six times compared with the average cell in the apical 2 mm. Erickson and Goddard have shown that there is an increase of about twice this value when the elongated cells are compared with the cells in the apical 1 mm only. The dry weight increases by nearly four times as new wall material, and new protoplasm, are laid down. The slight decrease in the rate of increase of dry weight noted in the 4–6 mm region (Fig. 2B) corresponds to the zones of maximum elemental rates of elongation and of increase in fresh weight. At this point elongation and fresh weight increase is probably brought about mainly by water uptake, and the volume increase is going on at a faster rate than the production of dry matter (25).

During the elongation process total nitrogen of the average cell more than doubles; the coefficient of increase shown in Table I agrees exactly with that derived from the data of Erickson and Goddard. Much of the increase in nitrogen can be attributed to the considerable augmentation of soluble nitrogen; Table I shows a coefficient of increase of 4.5. Morgan and Reith (33) found even greater increases in the broad bean.

It is agreed by most students that protein synthesis accompanies the elongation phase or most of it (3, 6, 8, 25), and in the present studies the protein nitrogen level has been shown to increase up to the 8–10 mm segment. The large increases in soluble nitrogen cannot result from protein degradation

in the elongating cells then, but probably result from amino acids and amides translocated from the endosperm and proximal regions of the root and deposited in the newly forming vacuoles.

The cells of the 8–10 mm segment contain only about 1.5 times the protein of the apical segment cells, but net protein synthesis is not the only indication of active protein metabolism. As in animal tissues, protein in the plant is thought to be in a state of dynamic equilibrium (45), and it is possible to have rapid turnover without any net synthesis. Steward, Bidwell, and Yemm (43) believe that in growing carrot tissue explants certain proteins are being turned over rapidly while others are relatively metabolically inactive. There is considerable evidence that protein is being rapidly changed in the region of elongation. Morgan and Reith (33) have shown that in broad bean roots the mitotic cells have a higher concentration of certain amino acids than the elongating cells, and reveal on chromatograms several unidentified ninhydrin-reacting substances not found in other regions. These differences applied to the amino acid constituents of several protein fractions as well as the free acids. More convincing evidence of protein turnover is seen, however, in the data on enzyme activity.

Enzyme Augmentation during Cell Elongation

Transaminase, and those enzymes studied by Brown, Robinson, and Cartwright (11, 36, 37, 38, 39), all increase during cell elongation with increase in protein, and reach a maximum between 8 and 12 mm from the tip. It has already been noted, however, that transaminase activity does not increase in direct proportion to increase in protein nitrogen. During elongation transamination per unit protein nitrogen nearly doubles, and Robinson and Brown found a considerable increase in the activities per unit protein nitrogen of dipeptidase, glycine oxidase, and invertase. They also found that each of their enzymes did not change to the same degree. It is evident that the rapidly elongating cells with their high rate of metabolism require a special enzyme complement and a larger proportion of specific protein than do the mitotic cells (11). The raw materials for the synthesis of these specific proteins come from the non-specific protein of the meristematic mother cells, and from free amino acids, amides, and other carbon chains translocated from the endosperm and from the more mature regions of the root where protein is decreasing, and which Brown and Wightman (13) have shown to be essential to the growth of the elongation region. The high concentrations of free amino acids which accumulate in the elongating cells are a storage of raw materials for synthesis of specific protein, and the sugar noted by deVries (46, 47, 48) is a ready source of energy, and possibly carbon chains for protein synthesis. Steward, Bidwell, and Yemm (42, 43) claim that in their growing carrot tissues the carbon of sugars is more accessible for protein synthesis than that of many of the free amino acids, such as glutamic and aspartic acids and threonine. The latter they regard as products of protein breakdown and as storage products and not as immediate precursors of protein. If this should prove to be the case in the growing radicle then we must look to

much of our pool of free amino acids and amides as a source of nitrogen only with its carbon being either respired or recycled via organic acids to carbohydrate.

It is a reasonably safe assumption that nearly all of the protein in the maximally extending cell is specific, but it has not been possible to determine what proportions of specific and non-specific (enzymatically inert) protein occur in the non-vacuolated cell. The glutamic–aspartic transaminase of the corn radicle has a coefficient of increase of 2.6 (Table I). Let us assume that this value represents the mean coefficient of increase of all enzymic protein. The coefficient of increase of total protein during elongation is 1.5. This value will vary with the size of the apical segment, but the 2 mm segment used will give a mean value for all of the mitotic cells. Taking this increment as an arbitrary unit it is apparent that 2 units of protein in the mitotic cell increase to 3 units in the fully elongated cell. The specific protein of the mitotic cell then, according to our assumptions, amounts to 1.2 units, or to three-fifths of the total protein. It seems reasonable that the non-specific protein in such a cell amounts to less than half the total when we consider the large size of the mitotic cell nucleus, and regard all of the protein of the nucleoplasm as being specific.

The coefficients of increase of phosphatase, dipeptidase, glycine oxidase, proteinase, ribonuclease, and desoxyribonuclease, read from the graphs of Brown and Robinson (11) and Robinson and Cartwright (39), are somewhat higher—near 4 for the first three, and 6 for the proteolytic and nucleolytic enzymes. If the values of these workers were based on the larger 2 mm sections, however, their coefficients would be lower, and probably closer to that of the transaminase.

Invertase is the one enzyme so far studied which increases during cell growth to an extent far in excess of any other. Its coefficient of increase (11, 37) is about 25. It is difficult to conceive that this tremendous increase in invertase activity is causally related to the growth process. Known functions do not assist an understanding of the differences in augmentation during elongation of two such enzymes as invertase and transaminase. An alternative view is that the unusually great increase in invertase activity is associated with its intracellular localization. The amount of increase of enzymes which are located in the interior of the cytoplasm will be limited by the extent of increase of the entire cell. Since growth from the non-vacuolated cell to the vacuolated cell is almost entirely a case of longitudinal extension, growth in mass, volume, and surface will have nearly similar coefficients of increase. According to the coefficient of increase of fresh weight (Table I) this value is slightly more than 6. It may be noted that neither transaminase nor any of Brown's enzymes, except invertase, have coefficients exceeding this value.

If, however, an enzyme were located on some part of the cell which increased very greatly during growth its coefficient of increase would exceed 6. The vacuole is absent, or is at least too small to be detected, in the mitotic cells,

and thus the coefficient of tonoplast increase must be of much greater magnitude than that of the interior mass of cytoplasm or of the ectoplast. By postulating that invertase is located, at least in part, on the tonoplast (or on some part of the cell that behaves similarly) it becomes possible to understand how it can increase 25-fold during cell elongation. It may be that invertase exerts some control over the diffusion of sugars across the tonoplast, thus playing an essential part in the process of vacuolation.

Time Course of Growth and its Functions

It was routinely determined on several populations that corn radicles of 6 to 10 mm length were growing at an average rate of 0.95 mm per hour. This agrees closely with the findings of other workers in this laboratory (24). If we take the average cell to be fully elongated by the time it is 8 mm from the tip, then the time taken to reach this stage from the non-vacuolated, mitotic condition is about 8 hours. The events which occur along the radicle axis as elongation proceeds can be expressed in terms of increase per unit time by using this growth rate. As an example, let us calculate the rate of protein synthesis.

From Fig. 4B it may be seen that the average mitotic cell contains 0.108 mμg protein nitrogen per cell, or 0.675 mμg protein per cell. This latter value is derived by multiplying the value for protein nitrogen by the conventional conversion factor 6.25. A line drawn through the mid-points of the histogram (Fig. 4B) shows that the protein is increasing at a uniform rate between the apical segment and the 8–10 mm segment. From the slope of this line it can be calculated that protein nitrogen per cell is increasing at the rate of 0.0067 mμg per hour, and protein at the rate of 0.042 mμg per hour. The per cent increase in protein is, therefore, $\frac{0.042 \times 100}{0.675} = 6\%$ per cell per hour.

Assuming as before that only three-fifths of the protein of the average mitotic cell is specific protein, whereas all that in the vacuolated cell is specific, it can be calculated that the rate of increase of enzyme protein is 0.063 mμg per cell per hour, or 16% per cell per hour.

Fresh weight per cell and dry weight per cell both increase at a constant rate up to the 4–6 mm segment. This rate amounts to 15 mμg fresh weight per cell per hour, and 1.2 mμg dry material per cell per hour. The corresponding percentage increases are 107% per cell per hour, and 55% per cell per hour, respectively.

The rate of increase of glutamic–aspartic transaminase activity continually decreases throughout the growing region (Fig. 4A). Between the first two segments the rate of increase is 43% per cell per hour, but from the 4–6 mm segment to the 6–8 mm segment the value has fallen to 11% per cell per hour.

The results presented in this way, as time rates, illustrate more graphically the high metabolic activity and rates of synthesis in elongating radicle cells. It will be noted that during the early stages of cell elongation the synthesis of transaminase is much greater than the synthesis of protein, or even of the

specific protein as a whole, although over the whole elongation region the coefficient of increase in transaminase is of the same order as that of specific protein.

The Decline in Metabolic Rates Following Elongation

There is little doubt that after or near the completion of cell elongation protein begins to decline, but there appears to be considerable variation in the exact region where it occurs. Some of this variation may be due to experimental error, but it is probable that specific differences are important as well. Variation between the three studies on corn may be attributed to varietal differences, or to the fact that the seedlings were not at the same stage of development. It has been pointed out above that the spatial and temporal sequences in the growth phenomena and metabolic reactions do not remain the same in the corn radicle as the tip moves further from the endosperm (24, 35).

Baldovinos (3), who found a decrease in protein nitrogen beginning about 3 mm back from the tip, does not state the age or length of the radicles on which the nitrogen was determined, but it appears from his photographs that they were less than 2 cm in length. Erickson and Goddard (25) found the decrease beginning at the 5 mm mark on radicles 2.5 to 3.0 cm in length. The experiments reported here were performed on radicles 3 days old and from 6 to 10 cm in length, and protein loss only occurred proximal to the 10–12 mm segment. Erickson and Goddard found in their material that the maximum elemental rate of protein loss was in the 6–7 mm segment.

Many enzymes so far studied show a decline on a cellular basis in the region proximal to elongation. Most of those studied by Brown and Robinson, phosphatase, dipeptidase, invertase, and glycine oxidase, have their maxima coinciding with, or just slightly proximal to, the maximum cellular protein level. Although the zone of decrease in transaminase activity per cell is further from the apex than the corresponding zones of the above four enzymes, its behavior is parallel in that the decrease follows the decline in protein level in the cells of the corn radicle.

With the proteolytic enzymes of broad bean radicle Robinson (36) has found that the initial increase per cell is relatively slow, and the maximum is not reached until the 11 mm region, about 3 mm proximal to the point of maximum protein level per cell. He suggests that the protein level is decreased by the high proteolytic enzyme activity, and that the level of these enzymes is one factor which controls growth. Data on soluble nitrogen in these more mature cells are rather meager, but Erickson and Goddard (25) found that total nitrogen remained constant after the protein level of the cell had begun to fall, which means that soluble nitrogen must have increased. This is also the region in which deVries (46, 47, 48) found high concentrations of amides. The available data show no evidence of a decline in fresh or dry weight per cell in any species studied.

Functions of Transaminase in Growth and Metabolism

By the very nature of the reactions they catalyze, it is apparent that the transaminases exert some influence on protein metabolism, but there is little

evidence that transamination occurs as an integral part of protein synthesis by a mechanism such as that suggested by Linderstrøm-Lang (30). It is true that Agren (1) has reported transamination between valylglycine and glycylaminobenzoic acid, and α-ketoglutaric acid, but his analytical methods are open to question, and Cammarata and Cohen (15) could find no evidence of activity with three peptides, leucylglycine, alanylglycine, and glycylglycine, or with methylglyoxal.

The changes in glutamic–aspartic transaminase activity per cell are very similar to the changes in glycine oxidase and dipeptidase activity in broad bean, and it is difficult to attribute some special role in protein synthesis to the transaminase that is not equally applicable to the other two enzymes, which are also involved with amino acids as substrates. It seems more probable that transamination is concerned with free amino acids and amides only, and that its influence on protein turnover is brought about by the maintenance of a proper amino acid balance (19). The investigations on the corn radicle suggest that such a control is exerted particularly at the sites of protein synthesis. Figure 4A shows that the maximum rate of increase in transaminase activity per cell occurs during the early stages of cell elongation, and a comparison of the Q_T(N) values in this figure with the protein nitrogen per cell of Fig. 4B shows that the amount of protein specific for transamination has reached a maximum value at least 3 mm distal to the region at which total protein per cell has reached a maximum. The transaminases are shifting the balance of free amino acids derived from the non-specific proteins of the mitotic cells, and the free amino acids and amides translocated from the endosperm and proximal regions of the root. If Steward *et al.* (42, 43) are correct in believing that many free amino acids, including glutamic and aspartic, are not the immediate precursors of protein, but only breakdown products, then we must alter our views on the role of the transaminases to some extent. In their carrot explants they visualize a condensation between nitrogen from nitrogen donors and carbon residues of sugars to form proteins directly in parts of the cell remote from the soluble reserves of amino acids. As yet we do not have evidence that this is the situation in plant tissues generally. It seems likely that glutamic and aspartic acids and their amides are at least important nitrogen donors in protein synthesis. In this case we might visualize the transaminases as converters of the products of protein hydrolysis to glutamic and aspartic acids utilizing α-ketoglutaric and oxaloacetic acids obtained from carbohydrate breakdown. These amino acids, or their amides, would then readily donate their nitrogen at centers of active protein synthesis. The resulting α-ketoglutaric and oxaloacetic acids would either be available for further transamination reactions, or enter the tricarboxylic acid cycle to be respired or converted to carbohydrate.

In the mitotic region Erickson and Goddard (25) have shown that the elemental rate of increase of protein corresponds with the elemental rate of increase of cell numbers, and presumably the lower rate of transaminase activity per cell in this region is sufficient to take care of the limited protein synthesis.

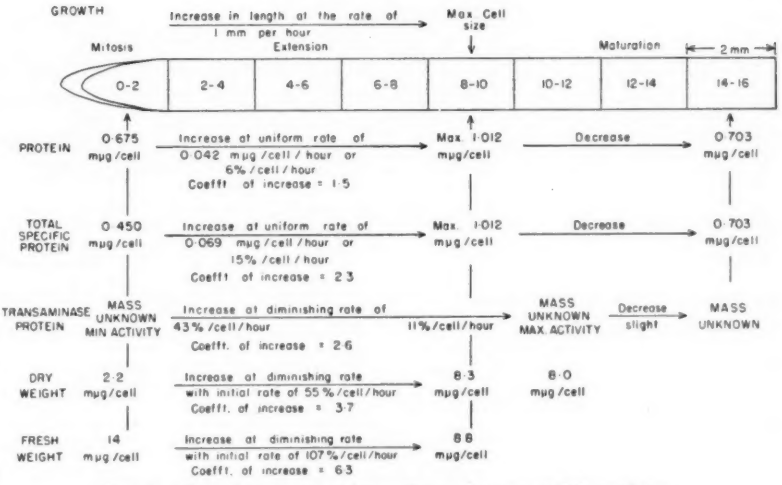

FIG. 5. Growth and generative cycles at the corn radicle meristem.

Although there is a decline in transaminase activity per cell after elongation, the activity is maintained at a much higher level than in the mitotic region. This activity is associated with protein hydrolysis brought about by the high proteolytic enzyme activity in a manner similar to the scheme suggested by Panalaks (34). In view of the high amide concentration in this region, it seems that many of the amino acids resulting from protein breakdown are deaminated by transamination to oxaloacetic and α-ketoglutaric acids, and the resulting aspartic and glutamic acids amidated to their corresponding acid amides. The ammonia required for amidation may come from those amino acids which do not take an active part in transamination, but rather are oxidatively deaminated. These amino acids and amides are then transported back to the growing region, and to a limited extent to the mitotic region, where they do not accumulate but are rapidly metabolized.

A cycle of nitrogen has thus been postulated which is presented schematically in Fig. 5. It is realized that such a scheme ignores such behavior as cell differentiation and is, no doubt, oversimplified, but it does present a reasonable hypothesis for which there is considerable evidence and upon which much further experimentation can be based.

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NOTES ON EXOBASIDIUM[1]

D. B. O. SAVILE

Abstract

The biology, taxonomy, and host relationship of *Exobasidium* are discussed. A key, detailed descriptions, and host records are given for *Exobasidium vaccinii* Wor., *E. vaccinii* var. *arctostaphyli* (Harkn.) comb. nov., *E. burtii* Zeller, *E. decolorans* Harkn., *E. canadense* sp. nov., *E. cordilleranum* sp. nov., *E. cordilleranum* var. *minor* var. nov., *E. vaccinii-uliginosi* Boud., *E. vaccinii-uliginosi* var. *phyllodoces* var. nov., *E. dimorphosporum* sp. nov., and *E. empetri* Ito & Otani. Evidence is presented to support the derivation of *Empetrum* from Ericaceae.

Introduction

Recent acquisition of many specimens of *Exobasidium* from various parts of Canada, but particularly British Columbia, has necessitated critical study of some taxa of this curious but exasperating genus. The confusion that surrounds the genus is due to a variety of causes. (i) In some species the morphology is decidedly variable, notably the septation of the basidiospores and the number of sterigmata on the basidium in *E. vaccinii* and a few related taxa. (ii) In some species, again notably *E. vaccinii*, the symptoms induced in the host are highly variable, although in others they are quite constant. (iii) Many early authors described species purely on the basis of host relationship without serious consideration of morphology. (iv) In some species there is abundant production both of secondary sporidia, i.e., ballistospores formed directly from the basidiospores but much smaller than them, and of acicular to ellipsoid conidia. Numerous species have been inadequately described with no mention of the basidia and with no clear indication whether the spores are basidiospores, secondary sporidia, or conidia. Such names must often be regarded as *nomina dubia* unless authentic material is available. (v) When overmature, some species may be overgrown by saprophytic or hyperparasitic molds, notably a delicate *Ramularia*. It is suspected that the spores of these secondary fungi have occasionally been included in the size range given for the basidiospores; such at least is the most charitable explanation for the departure of some descriptions from reality. (vi) Some host plants take two or more species of *Exobasidium*. This fact need cause no confusion when the species are as distinct as, e.g., *E. vaccinii* and *E. vaccinii-uliginosi;* but it may be misleading when the morphological difference is slight. (vii) In species such as *E. vaccinii*, in which conidia and secondary sporidia are common, it may be difficult to secure an adequate set of measurements from a single specimen; hence a long series of specimens is desirable for each host. (viii) Not enough is known of the effect of environment on symptom expression or the production of conidia and basidia. (ix) Not

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enough cross-inoculation studies have been made, particularly in the *E. vaccinii* complex. (x) Finally, not all the fungi ascribed to the genus belong there. Some are not even Basidiomycetes.

Under the circumstances complete and wholly reliable citation of synonyms is impossible. Under *Exobasidium vaccinii* I have given a conservative list of synonyms to which many names might justifiably be added. In particular I suspect that several species described on *Rhododendron* from various parts of the world may belong in this species. In some of these taxa the spores are described as being somewhat larger than those of *E. vaccinii*, and one or more varieties or segregate species may be recognizable; but in some instances examination of authentic material has shown the distinctions to be imaginary. Only careful study of authentic material of the various taxa involved would allow detailed synonymy to be presented.

When Burt (2) monographed the genus for North America, in a well-merited reaction against the haphazard naming that had prevailed for some forty years, he recognized only three species, *E. vaccinii*, *E. vaccinii-uliginosi*, and *E. symploci*. The last species, on *Symplocos tinctoria* (L.) L'Her. in the Gulf States, is known only from the conidial state, but is probably authentic, because other species are known on this genus in the Old World tropics. Burt suspected that the fungus on *Rhododendron albiflorum*, of which he had seen little material, might be distinct, and this fact was verified by Zeller, who named it *E. burtii*. Burt listed *E. decolorans* Harkn. as imperfectly known, no specimens on the type host, *Rhododendron occidentale*, being available to him. This is a good species, and an emended description is given in this paper, but Harkness' measurements were quite erroneous. Two other species have been described in North America since Burt's monograph: *Exobasidium parvifolii* Hotson, which is morphologically indistinguishable from *E. vaccinii* but produces curious shoe-string galls on *Vaccinium ovalifolium* and *V. parvifolium*, and which is discussed below under *E. vaccinii;* and *E. angustisporum* Linder on *Arctostaphylos alpina*, which is fully typical *E. vaccinii*. Nevertheless there are several other taxa, unknown to Burt or of which he saw inadequate material, that are clearly distinct from the species that he recognized; and these are presented below.

Taxonomic Position

The true position of *Exobasidium* within the Basidiomycetes is uncertain. Burt was quite satisfied to retain the genus within the Thelephoraceae, but few if any mycologists would agree to such a disposition today. Most authors now place it in Exobasidiaceae Schroeter (Pilze Schles. 1:413. 1889). Several genera have from time to time been added to the family, but probably only *Kordyana*, parasitic on Commelinaceae, should be included. It is necessary, however, to erect a new genus for the fungus on *Saxifraga*, described as *Exobasidium warmingii* Rostr., which is intermediate between *Exobasidium* and *Kordyana;* this organism is treated in a forthcoming paper (8). The family may be placed in the Heterobasidiomycetidae or Homobasidiomy-

cetidae according to one's definition of the limits of these two groups. I have previously (5) treated it as the most primitive of the Homobasidiomycetidae and nearly on the line of origin of this group from the Heterobasidiomycetidae. Talbot (9) would include it in the latter group, which he defines to include holobasidiomycetes in which the basidiospores germinate by repetition. Biochemistry may eventually throw light upon the position of the family.

Host Relationship

The host range of the genus *Exobasidium* cannot be designated with certainty without examination of authentic material of various fungi that have been ascribed to it. The recent recognition of a species on *Empetrum* has focussed interest upon the true host range. In addition to occurring abundantly on Ericaceae, the genus also definitely occurs on Theaceae, Lauraceae, and Symplocaceae; and *E. antarcticum* Speg. on *Lebetanthus* (Epacridaceae) seems from its description to be the conidial state of a species. It is significant that Theaceae, Ericaceae, Epacridaceae, and Empetraceae are members of what Gibbs (3) has termed the HCl/Methanol Positive Series. Professor Gibbs has not yet tested *Laurus* or *Symplocos*; but both are aromatic, glandular, and somewhat similar to Theaceae in some other respects, and it will be no surprise if they prove to belong to this highly natural series. The relationship of *Empetrum* to Ericaceae has long been suspected on morphological and ecological grounds, and the occurrence of a *Chrysomyxa* on *Empetrum* has been cited as further supporting evidence. *Chrysomyxa empetri* is very similar to *C. ledicola* on *Ledum*, so that the evidence is probably valid; but it should be noted that the mere occurrence of a *Chrysomyxa* on *Empetrum* is not decisive, for a prime requirement of this genus of rusts is the evergreen habit of its hosts; and one species occurs on *Ilex*, which is definitely not in this series of plants. It may further be noted that *E. empetri* is close to *E. vaccinii-uliginosi*, *E. cordilleranum*, and *E. dimorphosporum*, all on Ericaceae, which further suggests that *Empetrum* is derived from an ericaceous ancestor.

A number of *Exobasidia* have been described on various hosts unrelated to the series discussed above, including much more modern herbaceous species. It is conceivable that a few of these are valid, but experience suggests that it is improbable. The following notes and dispositions will serve as examples:

Exobasidium vitis (Viala & Boyer) Prill. & Delacr., on *Vitis*, is *Aureobasidium vitis* Viala & Boyer, a hyphomycete.

Exobasidium stellariae Syd., on *Stellaria*, is *Melampsorella caryophyllacearum* Schroet.

Exobasidium schinzianum Magn. in Sacc. on *Saxifraga* is *Entyloma schinzianum* (Magn.) Bubák.

Exobasidium warmingii Rostr., on *Saxifraga*. As stated above, this is related to but distinct from *Exobasidium* and *Kordyana* and it is being treated elsewhere (8). It may be noted that *Roum. F. Sel. 4815* (DAOM) is *Puccinia pazschkei* overrun by a delicate hyphomycete.

Exobasidium tradescantiae Pat. on *Tradescantia* is *Kordyana tradescantiae* (Pat.) Rac.

Exobasidium brevieri Boud. on *Asplenium* is *Herpobasidium filicinum* (Rostr.) Lind.

Exobasidium patavinum D. Sacc. on *Ilex*. The available isotype, *D. Sacc. Myc. It. 8* in DAOM, is a superficial saprophyte affin. *Pellicularia* on dead leaves.

Exobasidium celtidis T. S. & K. Ramak. on *Celtis*. Not seen but the broadly ellipsoid spores, irregularly to muriformly septate, are widely different from those of any valid *Exobasidium* familiar to me.

Exobasidium giseckiae Allesch. on *Giseckia* (Aizoaceae). Described as fruiting amphigenously and having 4-spored basidia with spores ellipsoid to ovoid, 6–9×4–5 μ. This might be *Pellicularia filamentosa* (Pat.) Rogers, which Rogers (5) recorded on such diverse hosts as *Boerhavia*, *Chimaphila*, *Citrus*, *Dianthus*, *Ficus*, *Hydrophyllum*, *Nicotiana*, *Solanum*, and an undetermined amaryllid. It unquestionably occurs on many other plants.

Exobasidium hesperidium Maire, on *Rhus*, was described as having the basidia fascicled in the stomata and the spores apparently becoming muriform.

Under the circumstances it is only prudent to withhold judgment on any species of *Exobasidium* described from families other than those listed above.

Materials and Methods

Unless otherwise specified the specimens studied are in DAOM. DAOM accession numbers are given when no collector's numbers are available. Specimens from the Forest Biology Laboratory, Victoria, B.C., in DAOM are given under the Victoria accession numbers (DAVFP). The following abbreviations have been used for collectors' names, C(alder), S(avile), F(erguson), P(armelee), T(aylor), V(aillancourt), in various combinations.

Although thin sections are necessary in studying the ontogeny of *Exobasidium* they seldom provide the numerous basidia and spores in a single mount that are desirable in taxonomic studies. Scrape mounts have accordingly been used in the study of most of the specimens examined in this study, as being much faster to prepare, more economical of material, and providing a concentrated mount. The following method has proved satisfactory. Three small drops of lactophenol containing about 0.01% cotton blue (much more dilute than is usually recommended) are placed on a slide at intervals of about 5 mm. The center drop should be very small and the others large enough to fill the space under the cover glass. Working under the dissecting microscope, selected areas of the hymenium are gently scraped with a fine diagonal scalpel slightly moistened with lactophenol (by dipping the tip into one of the drops on the slide and removing the surplus with filter paper or a finger tip). The scalpel should be honed to a smooth edge, e.g. on crocus cloth. Float the scraped material into the center drop and repeat until ample has accumulated. Place the cover glass in position and warm over a

very small flame, pressing on the cover if necessary to remove air bubbles; but be careful to avoid lateral movements of the cover, which often roll up the delicately extended scrapings into an unrecognizable mass. This method keeps the basidia and spores concentrated in the center of the drop. It is also excellent for spore mounts of rusts, smuts, and other fungi. Satisfactory mounts may be quickly ringed with clear nail lacquer.

This method does not yield the full length of the basidium, but cuts it off approximately where it emerges from the cuticle. Some cuticle is usually taken in the scraping and some basidia are, of course, cut off nearer the apex; but if the longer basidia are assumed to be cut off at the cuticle there will seldom be any serious error, and such an assumption has been made in the descriptions that follow. Actually the base of the basidium cannot always be located with any certainty in sections and the emergent lengths may yield better comparisons than inadequate measurements of full lengths. Basidium length is in any event often highly variable and is a less valuable taxonomic character than width, sterigma size, and sterigma number.

Key to North American Species of *Exobasidium*

This treatment excludes *Exobasidium symploci* Ell. & G. Mart., on *Symplocos tinctoria* (L.) L'Her. in the southern U.S. I have not seen this species, which is apparently known only from the conidia and hence is technically invalid although it probably belongs to this genus.

1. Basidiospores slenderly cylindrical to musiform.[2]
   2. Basidiospores 8.0–15.0(–18.0) μ long; basidia with (2–)3–6(–8) sterigmata; on various Ericaceae.
      3. Basidiospores 8.0–14.5×2.0–3.2 μ ..... *E. vaccinii* s. str.
      3. Basidiospores to 18.0 μ long and/or 5.0 μ wide ..... *E. vaccinii* s.l.
   2. Basidiospores 12.5–16.5(–20.0)×3.3–5.0 μ; basidia with 2–3–(–4) sterigmata ..... *E. vaccinii* var. *arctostaphyli*
   2. Basidiospores 14.0–24.0 μ long; causing leaf spots on deciduous-leaved *Rhododendron* spp.
      4. Basidiospores (14.0–)16.0–24.0×3.2–5.5 μ, strongly hooked at base, otherwise nearly straight, sterigmata 4(–5) ..... *E. burtii*
      4. Basidiospores 14.5–22.0×4.2–6.5 μ, ± evenly curved; sterigmata (2–)3–5(–6) ..... *E. decolorans*
      4. Basidiospores 14.0–20.0×3.0–4.7 μ, ± evenly curved; sterigmata 2–4(–6) ..... *E. canadense*
1. Basidiospores clavate or ellipsoid; on *Empetrum*, *Ledum*, or *Vaccinium*.
   5. Sterigmata 2–3(–4); conidia present.
      6. Basidiospores 14.0–19.0×3.5–6.3(–7.0) μ ..... *E. cordilleranum* var. *cordilleranum*
      6. Basidiospores (11.0–)13.0–16.5×3.0–4.5 μ ..... *E. cordilleranum* var. *minor*
   5. Sterigmata usually 2; conidia lacking.
      7. Basidiospores always continuous.
         8. Basidiospores 15.5–22.5×7.3–9.0(–11.5) μ ..... *E. vaccinii-uliginosi* var. *vaccinii-uliginosi*
         8. Basidiospores 13.0–17.0×4.8–6.0 μ ..... *E. vaccinii-uliginosi* var. *phyllodoces*
      7. Basidiospores frequently 1-septate.
         9. Basidiospores dimorphic, 13.0–18.5×6.5–8.5 or 18.5–28.5(–32.0)×5.0–7.0(–8.0) μ; sterigmata 5.0–7.0×1.5–2.5 μ ..... *E. dimorphosporum*
         9. Basidiospores uniform, 14–18×6–8 μ; sterigmata 8.5–11.0×3.0–4.5 μ. *E. empetri*

[2]Musiform = shaped like the fruit of a banana, i.e., slightly to moderately curved and tapering slightly toward each end; intermediate between falcate and allantoid.

Taxonomy

EXOBASIDIUM VACCINII Wor., Verhandl. Nat. Ges. Freib. 4: 397. 1867, var. VACCINII.

Fusidium vaccinii Fckl., Bot. Zeit. 19: 251. 1861 (*stat. conid.*).

Exobasidium azaleae Pk., Bull. Buffalo Soc. Nat. Hist. 1: 63. 1873; N.Y. State Mus. Rept. 26: 73. 1874.

Exobasidium discoideum Ell., Bull. Torr. Bot. Club 5: 46. 1874.

Exobasidium andromedae Pk., N.Y. State Mus. Rept. 29: 46. 1878.

Exobasidium cassandrae Pk., N.Y. State Mus. Rept. 29: 46. 1878.

Exobasidium ledi Karst., Grev. 42: 65. 1878.

Exobasidium oxycocci Rostr., Bot. Tidsskr. 14: 243. 1885.

Exobasidium discoideum Ell. var. *horvathianum* Thomas, Forstl.-Naturw. Zeitschr. 8: 305. 1897.

Exobasidium agauriae P. Henn., Engl. Bot. Jahrb. 28: 335. 1900.

Exobasidium karstenii Sacc. & Trott., Syll. Fung. 21: 420. 1912.

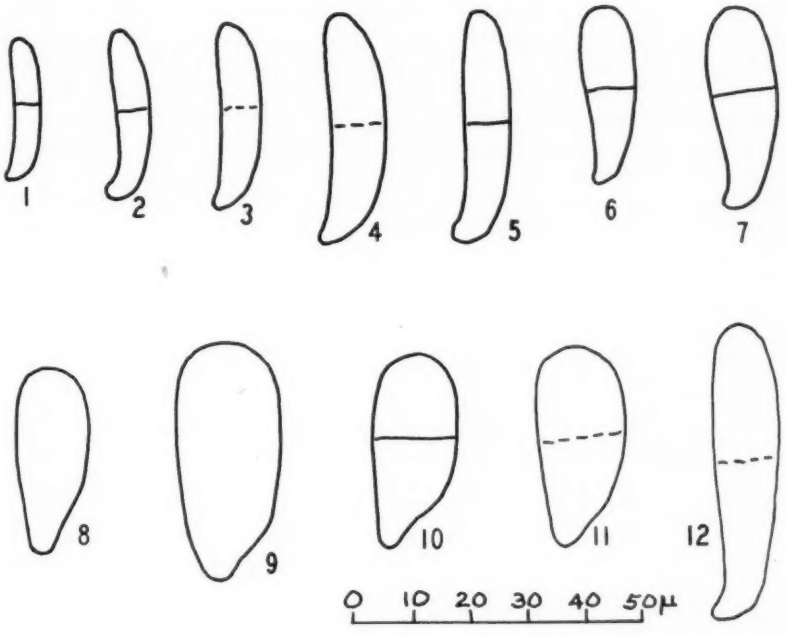
Exobasidium andromedae Karst., Thuem. Myc. Univ. 1110. 1878.

Exobasidium parvifolii Hotson, Phytopath. 17: 215. 1927.

Exobasidium angustisporum Linder in Polunin, Nat. Mus. Canada Bull. 97: 271. 1947.

Systemic, producing shoot galls or witches'-brooms; or localized, producing bud galls, leaf galls, or unhypertrophied leaf spots. Specimens on *Vaccinium vitis-idaea*, the type host, yield the following description: Basidia projecting 13–27(–30) μ beyond cuticle, 3.0–6.0(7.0) μ wide; sterigmata (2–)3–6(–8), 2.0–4.5×1.0–1.7 μ. Basidiospores 8.0–14.5×2.0–3.2 μ, nearly cylindrical, slightly to moderately curved, sometimes moderately hooked at base, (0–)1–(–3)-septate at maturity. Conidia often abundant, ca. 4.0–10.0×0.7–1.5 μ (Fig. 1).

The following specimens agree closely with the above diagnosis. On *Andromeda glaucophylla* Lk.: Lac Disparu, Kamouraska Co., Que., *Payette 894;* St. Anthony, Nfld., *S. & V. 2064.* On *Andromeda polifolia* L.: near Tow Hill, Graham I., Queen Charlotte Is., B.C., *C., S. & T. 21337A;* 18 miles west of Prince George, B.C., *C., S. & F. 14466;* Tyee, near Prince Rupert, B.C., *C., S & F. 13249;* Brabant I., Great Slave L., Mack., *Lewis 1010;* Fallis Bog, near Edmonton, Alta., *Conners DAOM 2488;* Chesterfield Inlet, Keew., *Savile & Watts 1135;* Great Whale River, Que., *Savile 513;* Finland, *Theum. Myc. Univ. 1110* (type of *E. karstenii*). On *Arctostaphylos alpina* (L.) Spreng.: Chesterfield Inlet, Keew., *Savile & Watts 1254.* On *Arctostaphylos uva-ursi* (L.) Spreng.: 5 miles southeast of Terrace, B.C., *C., S. & F. 13070;* near Houston, B.C., *C., S. & F. 13427;* Beaverdell, B.C., *Grant DA VFP 10050;* Malakwa, B.C., *C. & S. 8831;* near Kimberley, B.C., *C. & S. 11371;* 16 miles west of Banff, Alta., *C. & S. 12210;* Finland, *Myc. Fenn. 624.* On *Cassiope tetragona* (L.) D. Don: Four Rivers Bay, 72° 50′ N. 95° 30′ W., Somerset I., Frank., *Savile 3693;* Spence Bay, 69° 31′ N.: 93° 27′ W., Frank., *Savile 3897.* On *Chamaedaphne calyculata* (L.) Moench. (leaf galls only): Abbotsford, Que., *Savile DAOM 13847.* On *Kalmia polifolia* Wang.: near



FIGS. 1–12. Representative mature basidiospores of North American species and varieties of *Exobasidium*. Solid septum indicates general, and broken septum occasional, occurrence. Secondary septa, which occur irregularly, often only at germination, are omitted. Fig. 1. *E. vaccinii* var. *v.* Fig. 2. *E. vaccinii* var. *arctostaphyli*. Fig. 3. *E. canadense*. Fig. 4. *E. decolorans*. Fig. 5. *E. burtii*. Fig. 6. *E. cordilleranum* var. *minor*. Fig. 7. *E. cordilleranum* var. *c.* Fig. 8. *E. vaccinii-uliginosi* var. *phyllodoces*. Fig. 9. *E. vaccinii-uliginosi* var. *v.-u.* Fig. 10. *E. empetri*. Figs. 11, 12. *E. dimorphosporum*.

Summit L., Hart Hwy., B.C., *C., S. & F. 12492*. On *Rhododendron viscosum* (L.) Torr.: Newfield, N.J., *Thuem. Myc. Univ. 210* (type of *E. discoideum*); ditto, *Roum. F. Sel. 4545*. On *Rhododendron macrophyllum* G. Don (*R. californicum* Hook.): Humboldt. Co., Calif., *Calif. Fungi 454*. On *Vaccinium angustifolium* Ait.: Constance Bay, Ont., *Shields DAOM 31082*. On *Vaccinium corymbosum* L.: Cartyville, Nfld., *Hockey DAOM 40544*. On *Vaccinium macrocarpon* Ait.: Waverley, Mass., 1 Aug. 1908, *Seymour*. On *Vaccinium myrtilloides* Michx.: 9 miles southeast of Prince George, B.C., *C., S. & F. 12395;* Kingsmere, Gatineau Co., Que., *Conners DAOM 4526;* Danford L., Gatineau Co., Que., *Savile DAOM 19574;* Cloud L., Kings Co., N.S., *Ward DAOM 3064*. On *Vaccinium myrtillus* L.: Germany, *Petrak Myc. Univ. 1730* (as *E. myrtilli*). On *Vaccinium* affin. *ovalifolium* J. E. Sm. (systemic): 8½ miles east of Hope, B.C., *C. & S. 10499*. On *Vaccinium ovalifolium* J.E. Sm. (shoe-string galls = *E. parvifolii*): mountain south of McLinton Cr., Graham I., Queen Charlotte Is., B.C. *C., S. & T. 21599*. Ditto (unhypertrophied leaf spot): ½ mile north of Skidegate, Graham I., Queen Charlotte

Is., B.C., *C., S. & T. 21701;* near Justkatla, Graham I., Queen Charlotte Is., B.C., *Taylor 33.* On *Vaccinium ovatum* Pursh: Trinidad, Humboldt Co., Calif., 12 July 1933, *Bonar.* On *Vaccinium oxycoccos* L.: Mt. Hobbs, Athlow Bay, Graham I., Queen Charlotte Is., B.C., *C. & S. 21535;* Great Whale River, Que., *Savile 348;* St. Anthony, Nfld., *S. & V. 2020.* On *Vaccinium parvifolium* J. E. Sm. (shoe-string galls = *E. parvifolii*): Terrace, B.C., *Ziller DAOM 31854;* Healy, Vancouver I., B.C., *Ziller DAVFP 7688.* On *Vaccinium pennsylvanicum* Lam.: Nipigon River, Ont., 30 June 1884, *Macoun;* Norway Bay, Pontiac Co., Que., *Conners DAOM 2651;* Danford L., Gatineau Co., Que., *Savile DAOM 19574.* On *Vaccinium scoparium* Leiberg: Lumby, B.C., *Ziller DAVFP 9723.* On *Vaccinium uliginosum* L.: Finland, *Myc. Fenn. 629* (issued as *E. vaccinii-uliginosi*). On *Vaccinium vitis-idaea* L.: 3 miles west of Tlell, Graham I., Queen Charlotte Is., B.C., *C., S. & T. 23488;* Hudson Hope, B.C., *Groh DAOM 6725;* St. Anne de la Pocatiere, Que., *Payette 800;* Kelly's Camp, Gaspé Co., Que., *Riley DAOM 4597.*

The following specimens deviate slightly from the strict concept of *Exobasidium vaccinii*, the more important distinctions being shown in parentheses after the host. Some of these are probably valid varieties, but inadequate specimens and lack of host susceptibility data make it inadvisable to segregate them. The data are presented to make mycologists aware of the problems that still await their attention. On *Arbutus menziesii* Pursh (basidiospores 11–19 μ long): Deep Cove, Vancouver I., B.C., *Jones DAOM 8955.* On *Cassiope mertensiana* (Bong.) G. Don (sterigmata usually 2–3, ? = *E. cassiopes* Pk., N.Y. State Rept. 45: 83. 1893): Mt. Arrowsmith, Vancouver I., B.C., *C., P. & T. 19486;* Garibaldi, B.C., *Eastham DAOM 5957, Touzeau 8987;* Mt. Fougner, near Bella Coola, B.C., *C., P. & T. 20383.* On *Chamaedaphne calyculata* (L.) Moench. (basidiospores 11.0–17.5×2.8–5.5 μ; leaf spot or shoot infection; for leaf galls see above): Rivière Ouelle, Kamouraska Co., Que., *Payette 817*; St. Simeon de Bonaventure, Que., *Conners DAOM 12116;* Anticosti I., Que., *Adams DAOM 903;* St. Anthony, Nfld., *S. & V. 2558;* Whitewah, Trinity Bay, Nfld., *Hockey DAOM 39882.* On *Gaylussacia baccata* (Wang.) K. Koch (basidiospores 11.0–18.0×2.8–4.8 μ, also smaller, apparently secondary spores): Constance Bay, Ont., *Whetzel DAOM 18159;* Ste. Damase, Ste. Hyacinthe Co., Que., *Cinq-Mars DAOM 53023;* Nova Scotia, *Hockey DAOM 4562.* On *Ledum groenlandicum* Oeder (basidiospores 14.0–17.0 μ long, leaf gall only): 16 miles west of Banff, Alta., *C. & S. 12212.* On *Menziesia ferruginea* J. E. Sm. (basidiospores 11.5–18.5×3.0–4.0 μ): Falkland, B.C., *Ziller DAVFP 7808;* Monashee Pass, B.C., *C. & S. 10939.* On *Rhododendron macrophyllum* G. Don (basidia 7.0–9.0 μ wide, basidiospores 9.0–18.5 μ long): Manning Park, B.C., *C. & S. 10953B.*

There are unquestionably other minor morphological variants in other parts of the world, notably on *Rhododendron*, that might well be treated as varieties of *E. vaccinii.* A number of them have been named as species, but without careful study of abundant authentic material a sound disposition is impossible.

I am unable to see the basis for Linder's segregation of *E. angustisporum* from *E. vaccinii*, particularly as Linder in the same publication lumped together smut fungi that differed conspicuously in morphology as well as occurring on different hosts. It will be noted that the curious shoe-string galls that Hotson described as due to *E. parvifolii* are here assigned to *E. vaccinii* on the basis of the morphology of the parasite. This may be a specialized form of the fungus, but it will be noted that *E. vaccinii* also produces a leaf spot on *V. ovalifolium;* and the galls may be only a peculiar symptom expression.

Exobasidium vaccinii Wor. var. **arctostaphyli** (Harkn.) Savile comb. nov. *Exobasidium arctostaphyli* Harkn., Bull. Calif. Acad. Sci. 1: 30. 1884.

Exobasidium arctostaphyli was described by Harkness as having spores 10–12×4–5 μ, but Linder (4) illustrates spores about 15–20×4–5 μ in "authentic material on *Arctostaphylos pungens* from Harkness". It thus seems that Harkness may have recorded the spore lengths erroneously as he did for *E. decolorans*. Linder also noted that the basidia were usually 2- to 3-sterigmate. Most of the available material on *Arctostaphylos* spp. is referable to *E. vaccinii*, but two British Columbia specimens on *A. uva-ursi* (L.) Spreng. approach Harkness' fungus described from *A. pungens* H.B.K. in California: Kootenay Bay, B.C., *Eastham DAOM 6758;* Mt. Pope, near Fort St. James, B.C., *C., S. & F. 13797B*. In these specimens, both systemic, the sterigmata are 2–3(–4) and the mature basidiospores are 12.5–16.5× 3.3–5.0 μ, usually 1-septate (Fig. 2). In a few other specimens the spores are slightly wider than is usual for *E. vaccinii*. In view of the evident intergradation it seems necessary to reduce the Harkness taxon to varietal rank.

The following specimens on other hosts are apparently referable here. On *Cassiope tetragona* (L.) D. Don: Hudson Bay Mtn., near Smithers, B.C., *C., S. & F. 14647*; Rocher Deboulé Range, near Hazelton, B.C., *C., S. & F. 15152*. Note, however, that, in the arctic, *C. tetragona* harbors var. *vaccinii*.

Exobasidium cordilleranum Savile sp. nov.

Maculas pallidas ca. 4–8 mm diam. sine hypertrophia format in foliis. Basidia per cuticulum (14–) 19–31 μ exstantes, 3.5–7.5 μ lat.; sterigmata 2–3(–4) in basidio, 4.5–6.0 μ long., 1.3–1.8 μ lat. Basidiosporae 14.0–19.0 ×3.5–6.3(–7.0) μ, clavatae, 0–1(–3)-septatae, hilo manifeste hamato. Conidia 3.5–10.0(–14.0)×0.8–2.0 μ, aciculares (Fig. 7).

On leaves of *Vaccinium parvifolium* J.E. Sm.: near Empire Anchorage, Athlow Bay, Graham I., Queen Charlotte Is., B.C., *C. & S. 21444A* (**Type**); near Skidegate, Graham I., *C. & S. 21420;* Youbou, Vancouver I., B.C., *Ziller DAVFP 4610*. On *Vaccinium ovalifolium* J. E. Sm.: Aleza Lake, B.C., *Grant & Cottrell DAVFP 9269;* Robson Park, B.C., *Sanson 30;* Arthur L., Falkland, B.C., *Ziller DAVFP 7812;* near Nanaimo, B.C., *Krajina et al. 4908*.

The spores tend to be slightly smaller on *V. ovalifolium* than on *V. parvifolium*, but from the available series it is uncertain whether the difference is

real. Four-spored basidia also seem to be commoner on *V. ovalifolium* than on the type host.

Exobasidium cordilleranum is more or less intermediate in spore size, basidium size, and number of sterigmata per basidium between *E. vaccinii* and *E. vaccinii-uliginosi*. The habit, the frequently septate spores, and the production of conidia separate it sharply from the latter species.

Exobasidium cordilleranum Savile var. **minor** Savile var. nov.

A varietate typica differt basidiis ca. 20 μ exstantibus, 4.0–6.3 μ lat., basidiosporis (11.0–)13.0–16.5×3.0–4.5 μ, 0–1-septatis, hilo modice hamato (Fig. 6).

On *Vaccinium membranaceum* Dougl.: Falkland, B.C., *Ziller DAVFP 7809* (*DAOM 44106*, **Type**); Targhee Pass, Idaho–Montana border, July 1956, Parks *s.n.* (issued as on *V. caespitosum* but almost certainly *membranaceum*).

This variety approaches *E. vaccinii* in spore size, but the clavate spores and the preponderance of basidia with three sterigmata indicate its affinity with *E. cordilleranum*.

EXOBASIDIUM BURTII Zeller, Mycol. 26: 294. 1934.

Forming pale, unhypertrophied, circular leaf spots usually 5–15 mm diam.; basidia projecting 36–50 μ beyond cuticle, 8.0–10.0 μ wide; sterigmata 4(–5), 5.0–6.5×1.5–1.7 μ; basidiospores (14.0–)16.0–24.0×3.2–5.5 μ, 0–1(–3)-septate, straight or slightly curved but strongly hooked at base and thus with laterally projecting hilum; small secondary spores occasionally seen; acicular or ellipsoid conidia (3.0–)4.5–14.0×0.8–1.3 μ, often abundant (Fig. 5).

On *Rhododendron albiflorum* Hook.: 4 miles north of Nation R., on road to Manson Creek, B.C., *C., S. & F. 13757;* 12 miles north of Barkerville, B.C., *C., S. & F. 14325;* Frosty Mtn., Manning Park, B.C., *C., S. & F. 15727;* Mt, Revelstoke, B.C., *C. & S. 15823;* near Banff, Alta., *Sanson s.n.* (Also several immature specimens throughout southern B.C., taken early in the season.)

Burt, who noted this species as probably distinct, and Zeller, who described it, emphasize that the basidia are four-spored. This seems to be generally true, but several basidia with five spores were seen in the specimen from Banff. Possibly there is a distinctive population on the east slope of the Rockies; but intact basidia are hard to find in some specimens, and there may not be any real difference.

This and the next two species form a natural group, each being confined to a single deciduous-leaved species of *Rhododendron*. *E. magnusii* Woronichin (Monit. Jard. Bot. Tiflis 28: 18. 1913) on the deciduous-leaved *R. luteum* Sweet (*R. flavum* G. Don; *Azalea pontica* L., not *R. ponticum* L.) is evidently in this group of species. Two specimens have been seen: Kvischety, near Tiflis, *Kuske s.n.* (BPI); Sotschi, Transcaucasus, *Woronichin s.n.* (BPI). The first of these is overmature and the second bears conidia predominantly; but enough basidia and spores have been found to confirm Woronichin's description and to show that it is distinguishable from the three North American species: basidia, length doubtful, 6.2–11.0 μ diam., sterigmata 2–5, 2.5–3.5×1.4–2.2 μ. Basidiospores 14.5–21.0×4.2–4.8 μ, obscurely

0–1(–3)-septate, cylindrical to slightly clavate, straight or very slightly curved, hilum generally inconspicuous (but often on a laterally projecting base in immature spores). Small secondary sporidia present. Acicular conidia abundant, 4.5–9.0(–12.0)×0.7–1.4 μ. Producing pale leaf spots 1–4 mm diam.

EXOBASIDIUM DECOLORANS Harkn., Bull. Calif. Acad. Sci. 1: 31. 1884.

Forming pale, unhypertrophied, circular leaf spots usually 5–17 mm diam. Basidia projecting ca. 18–35 μ beyond cuticle, 6.5–8.5(–10.0) μ wide; sterigmata (2–)3–5(–6), 3.5–5.0(–8.0)×1.8–2.5 μ. Basidiospores 14.5–22.0×4.2–6.5 μ, continuous or tardily 1–3-septate, uniformly curved throughout length. Small secondary spores often seen. Conidia (6.0–)9.0–14.0(–17.0)×0.8–1.5 μ, acicular or narrowly ellipsoid, common (Fig. 4).

On *Rhododendron occidentale* Gray (all California): Lagunitas, Marin Co., *Harkness 2887* (CAS, **Type**); Smith R., Del Norte Co., *Parks 4368, 5191.* Ditto, *Cooke, Mycob. of N.A. 376;* Smith R., below South Fork, *Parks, s.n., UC M65098;* Old Village, Yosemite Nat. Park, *UC 734012;* Idyllwild, Los Angeles Co., Plunkett *UC 700180. Calif. Fungi 635* is presumably this species but both DAOM and UC packets are sterile, apparently immature.

Harkness described the spores as 7–8×4–5 μ, most improbable proportions; but the type, although meager, yields a few mature spores 19–22 μ long. Had the real dimensions been available to Burt he would doubtless have realized the affinity of this fungus with that on *R. albiflorum.*

Exobasidium canadense Savile sp. nov.

Maculas pallidas ca. 3–9 mm diam., sine hypertrophia, format in foliis. Basidia per cuticulum 24–40 μ extantes, 5.5–8.0(–9.0) μ lat.; sterigmata 2–4(–5), saepius 3, in basidio, 2.5–4.5×1.5–2.0 μ. Basidiosporae 14.0–20.0×3.0–4.7 μ, continuae vel denique 1–3-septatae, leniter vel modice et aequabiliter curvatae. Sporidia secundaria abundantia. Conidia 4.0–13.0×0.8–1.5 μ, aciculares (Fig. 3).

On *Rhododendron canadense* (L.) Torr.: North Woodstock, N.H., *Rel. Farl. 321a* (DAOM, **Type**); St. Simeon, Que., *Parent DAOM 7969;* Bartibog, N.B., *Davidson & Redmond DAOM 52192;* Caraquet I., Gloucester Co., N.B., *Dore et al. DAOM 23677;* Miscouche, P.E.I., *Conners DAOM 7744;* Canard, Kings Co., N.S., *Creelman DAOM 26766;* Oxford, N.S., *Hockey DAOM 4507;* Ecum Secum, Guysborough Co., N.S., *Savile 3250;* Berwick, N.S., *Conners DAOM 4983.*

Despite their evident affinity these last three taxa are consistently distinguishable and appear to be full species. In *E. burtii* the spores are almost straight for most of their length, but are strongly hooked at the base, whereas in *E. decolorans* they are nearly uniformly curved. The latter may be compared with bananas from the outside of a bunch, whereas the form of the *burtii* spore is that of the nearly straight banana usually seen in the center of a bunch. *E. canadense* also has uniformly curved spores, but they are slightly shorter and substantially narrower than those of *E. decolorans.*

The three species also differ in the range or predominant number of sterigmata. *E. decolorans*, with sterigmata generally 3–5 but occasionally 2 or 6, is perhaps closest to the ancestral species, which we may assume to have been very similar to *E. vaccinii*.

EXOBASIDIUM VACCINII-ULIGINOSI Boud., Bull. Soc. Bot. Fr. 4: 244. 1894. ? *Exobasidium aequale* Sacc., Nuovo Giorn. Bot. It. 24: 33. 1917.

Specimens on *Vaccinium uliginosum* yield the following description: systemic in shoots, causing infected leaves to be large, flat, and red above; fruits evenly over the lower leaf surface; basidia projecting ca. 25–32 μ beyond cuticle, ca. 9.0–10.0 μ wide, with 2 (rarely 3) sterigmata to 7.0 μ long $\times$ 1.8–2.5 μ wide at base; basidiospores 15.5–22.5$\times$7.3–9.0(–11.5) μ, broadly clavate to ellipsoid with hilum bluntly rounded and not prominent, never septate; conidia lacking (Fig. 9).

Exobasidium aequale on *V. myrtillus* from Italy was distinguished by Saccardo on the basis of the color and shape of the affected leaves. Specimens on this host from Sweden show somewhat greater variability than those on *V. uliginosum*, the spores ranging from nearly cylindrical to broadly ellipsoid with every intermediate form and some spores being irregular in outline, and the range of dimensions being 13.5–22.5$\times$5.0–9.8 μ. The basidia seen were about 6.0–6.5 μ wide and the sterigmata 4.5–4.8$\times$1.5–1.8 μ. Unless a long series of specimens should show these small differences to be constant it seems best to treat this fungus as a synonym of *E. vaccinii-uliginosi*.

Specimens on *Ledum groenlandicum* yield basidia 20–31 μ (free portion) $\times$ 5.8–9.5 μ, with sterigmata 3.2–6.0$\times$1.6–3.0 μ, and spores (14.0–)17.0–22.0 (–23.5)$\times$6.0–8.0 μ, practically embracing the combined range of variation on the last two hosts.

This species is distinguished in particular by its large and uniformly continuous spores, but also by the large, disterigmate basidia and lack of conidia. The symptom expression is also uniform in all hosts examined, fleshy galls and localized leaf spots never being produced. It should be noted that *Myc. Fenn. 629* on *Vaccinium uliginosum* is the leaf-spot phase of *E. vaccinii*, and that *Myc. Fenn. 628* is apparently hypertrophy due to mite or insect action (the curling rather than flattening of the affected leaf areas is the reverse of the typical *Exobasidium* reaction). Burt (2) pointed out that the original description of the spores of *E. vaccinii-uliginosi* as 25–32$\times$8–12 μ is erroneous. The stated maximum width is rarely approached, but the stated range of lengths does not even overlap that actually encountered.

Specimens examined.—On *Ledum groenlandicum* Oeder: Great Whale River, Que., *Savile 516;* St. Anthony, Nfld., *S. & V. 2099, 2218.* On *Vaccinium myrtillus* L.: Sweden, *Petrak Myc. Univ. 638* and *Nannfeldt 4521.* On *Vaccinium uliginosum* L.: Chesterfield Inlet, Keew., *Savile & Watts 1443;* Kelly's Camp, Gaspé, Que., *Riley DAOM 43988;* ? St. Anthony, Nfld. (immature), *S. & V. 2450;* Sweden, *Petrak Myc. Univ. 729.* On *Vaccinium vitis-idaea* L.: ? *Petrak Myc. Univ. 637* (immature, but wide basidia present).

Exobasidium vaccinii-uliginosi Boud. var. **phyllodoces** Savile var. nov.

A varietate typica differt basidiis angustioribus, 5.0–6.0 μ lat., et basidiosporis parvioribus, 13.0–15.5(–17.0)×4.8–6.0 μ (Fig. 8).

Specimen examined.—On *Phyllodoce empetriformis* (J. E. Sm.) D. Don: Skyline Trail, Yoho Nat. Park, B.C., *C. & S. 12147* (**Type**).

The leaves of the systemically infected shoots are longer and slightly wider than normal and are very pale. The paleness is surprising in view of the occurrence of anthocyanin in the flowers.

EXOBASIDIUM EMPETRI S. Ito et Otani, Trans. Myc. Soc. Japan 8: 3. 1958. (Mycol. Flora Japan 2, 4: 47. 1955, Japanese only).

Canadian material yields the following description: Systemic in vegetative shoots, causing infected leaves to become large, flat (maximum 8.0×2.8 mm) and deep red; fruiting on lower surface of leaf; basidia projecting 28–40 μ, 6.0–8.0 μ wide, with generally two erect or slightly divergent sterigmata 8.5–11.0 μ long and 3.0–3.5(–4.5) μ wide at base; basidiospores 14.0–18.0× 6.0–8.0 μ, generally 1-septate at maturity, rarely continuous or 3-septate, broadly clavate or ellipsoid, with conspicuous hilum usually projecting laterally. Conidia apparently lacking (Fig. 10).

Specimens examined.—On *Empetrum nigrum* L. (s.l.): Newton Point, southwest Graham I., *C., S. & T. 22951*, and alpine lake, 52° 55′ N. 132° 03′ W., Moresby I., *C. & T. 23130*, both Queen Charlotte Is., B.C.

The original description of *E. empetri* on *Empetrum nigrum* var. *japonicum* states that the affected leaves are yellow, rather than red, which may be due to genetic differences in the hosts. The spores are also stated to be 8.5–14.0 ×5.5–9.5 μ; but the proportions shown in the illustrations suggest that, if the widths are stated correctly, the maximum lengths must be substantially over 14 μ. There may be slight distinctions between the Japanese and Canadian fungi, but thorough study of abundant material would be needed to decide the point. Unfortunately the few specimens are too scanty to allow full assessment of the natural variability.

This species is of unusual interest both biogeographically and from the host relationship viewpoint. The curious distribution pattern in Japan and Sakhalin and otherwise apparently only in the Queen Charlotte Is. might be dismissed as due to inadequate collecting were it not repeated in a diversity of organisms; either identical or related species of bryophytes, ferns, and flowering plants show the same pattern, which will be discussed in due course by Mr. J. A. Calder; and Bousfield (1) has shown that an amphipod, *Paramoera carlottensis* endemic to the western Queen Charlottes, has its nearest relatives in Japan. This is one of several pieces of evidence that indicate that the summits of the Queen Charlotte Range and possibly much of the western slopes escaped glaciation at least during the last advance of the Cordilleran ice sheet. The significance of *E. empetri* as evidence of the relationship of Empetraceae and Ericaceae has been discussed under Host Relationship.

Exobasidium dimorphosporum Savile sp. nov.

Ramulos infecit et folia leviter deformat. Laminas totas foliorum occupat. Hymenium hypophyllum. Basidia per cuticulum 26–40 μ exstantes, 5.0–9.5 μ lat.; sterigmata 2, raro 3, in basidio, 5.0–7.0×1.5–2.5 μ. Basidiosporae dimorphae, vel 13.0–18.5×6.5–8.5 μ et hilo inconspicuo vel 18.5–28.5(–32.0) ×5.0–7.0(–8.0) μ et hilo manifeste hamato, 0(–1)-septatae. Conidia mihi ignota sunt (Figs. 11, 12).

On *Vaccinium caespitosum* Michx., between Baldy Mtn. and Dunn Peak, 6800–7300 ft, east of North Thompson River, B.C., *C., P & T. 19882* (**Type**). Isotypes in BPI, G, IARI, IMI, RM, TRTC, UC, UPS.

Both the habit and the morphology of this species indicate its affinity with *Exobasidium vaccinii-uliginosi*. It is distinguished by the dimorphic and frequently septate basidiospores. The broad spores fit *E. vaccinii-uliginosi* in width but are somewhat too short; whereas the narrow ones are both too narrow and too long for that species. I have seen no other specimens of this fungus, but it is possible that material is on file in some herbaria under *E. vaccinii* or *E. vaccinii-uliginosi*.

Phylogeny

Anything approaching an adequate understanding of the phylogeny of such an anomalous and seemingly isolated genus as *Exobasidium* is probably impossible. Nevertheless it is possible to draw some tentative conclusions from a study of the North American taxa. The amount of host specialization, unaccompanied by morphological distinctions, that may occur in *E. vaccinii* is still open to doubt, although field observations suggest that it may be substantial. Nevertheless this species, defined morphologically, has a wide host range. *E. vaccinii* is also unusually unstable in morphology, the number of sterigmata commonly ranging from 3 to 6 and occasionally from 2 to 8 in a single specimen. It therefore seems to be a plastic species and may be close to the ancestral stock from which the remaining taxa attacking Ericaceae arose. Some of the slightly aberrant specimens listed above under *E. vaccinii*, and some of the species described, especially on *Rhododendron* spp. from other continents, may represent incipient species segregating from *E. vaccinii*.

At least two evolutionary lines seem to have emerged from the *E. vaccinii* complex. One line has led to the group of related species that form unhypertrophied leaf spots on deciduous-leaved *Rhododendron* spp., *E. magnusii*, *E. canadense*, *E. decolorans*, and *E. burtii*. In this group there has been substantial increase in the size of the basidiospores, with little change in shape, and some degree of stabilization in the number of sterigmata. In the other evolutionary line the trend has been to reduction in number of sterigmata, increase in size of basidiospores, change of basidiospore shape to ellipsoid or clavate, elimination of conidia, and perhaps elimination of secondary sporidia. This line has arisen through forms close to *E. vaccinii* var. *arctostaphyli* and *E. cordilleranum* var. *minor*. The tentative relationship of these various taxa is shown in Fig. 13.

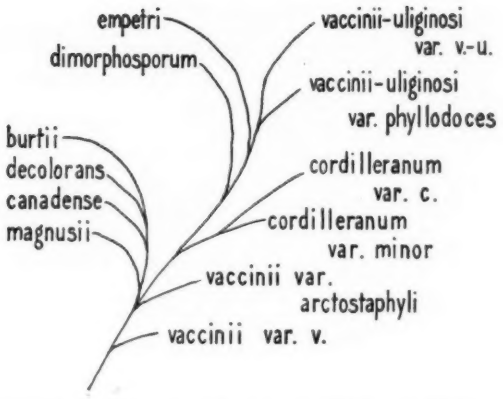


FIG. 13. Tentative evolutionary diagram of the species and varieties of *Exobasidium* attacking Ericaceae and Empetraceae.

In the second and larger evolutionary line there is indication of simplification of the life cycle as well as increasing host specialization. The preponderance of disterigmate basidia in the advanced taxa suggests that there may also have been a simplification of the nuclear behavior. It is probable that in the regularly disterigmate taxa there is no normal meiosis and that some form of apomixis prevails; and it is possible that some of those with large basidiospores have diploid nuclei.

There is an apparent parallel between the evolution of *Exobasidium* and that of *Chrysomyxa*, which also occurs predominantly on Ericaceae and is presumably of approximately the same age. In *Chrysomyxa* the basic species may be taken as *C. ledi.* This species has small aeciospores and urediniospores, with shallowly sculptured walls, and occurs in at least six varieties with slight morphological differences on various species of *Rhododendron*, *Ledum*, *Chamaedaphne*, and *Vaccinium* (7). There are also several species with simplified life cycles (the only one with aecia having large and strongly sculptured aeciospores), and several segregate full-cycle species on *Rhododendron*, *Ledum*, or *Empetrum* with large and strongly sculptured aeciospores and urediniospores. It is interesting that *Ledum* and *Empetrum* bear advanced and closely related segregate species of both *Exobasidium* and *Chrysomyxa;* and it seems probable not only that *Empetrum* is derived from Ericaceae but also that it may have sprung from a source close to *Ledum.*

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THE TAXONOMY AND NOMENCLATURE OF THE WHEATS, BARLEYS, AND RYES AND THEIR WILD RELATIVES[1]

WRAY M. BOWDEN

Abstract

The correct name for the tribe that contains the wheats, barleys, and ryes and their wild relatives is TRITICEAE Dumort. The genus *Triticum* L. emend. is treated to include both the diploid, allotetraploid, and allohexaploid wheats (*Triticum* L.), and the diploid species and the allotetraploid and allohexaploid species of interspecific hybrid origin that were formerly placed in the genus *Aegilops* L. In the genus *Triticum* L. emend., there are 10 diploid species; one allotetraploid wheat species of hybrid origin (*T. turgidum* L. emend.) which includes many cultivars, three botanical varieties, and one auto-allohexaploid form; one allohexaploid wheat (*T.* × *aestivum* L. emend.) which is a hybrid complex that includes many cultivars; 10 other allotetraploid or allohexaploid species of interspecific hybrid origin; and numerous other artificial and natural interspecific hybrids. Section HORDEUM of the genus *Hordeum* L. contains the cultivated barleys and their wild relatives which are all classified under one species, *H. vulgare* L. emend. The taxonomy of the ryes and the genus *Secale* L. is considered briefly.

Introduction

In 1956, research studies by several different groups of Canadian cytogeneticists indicated an urgent need for more accurate information on the scientific names of species and interspecific hybrids in the wheats, barleys, ryes, and their wild relatives. I was asked to undertake a program of cytotaxonomic studies that in time would supply some of the needed information. The present paper is a report on the taxonomic situation in these cereals. Some problems are now solved, but much more cytotaxonomic research needs to be done in the future. The taxonomic problems in the barleys (*Hordeum* L. sect. HORDEUM) and in the ryes (*Secale* L.) are not as difficult to solve as the problems in the wheats and their relatives (*Triticum* L. and *Aegilops* L.). The latter are more difficult to understand and more investigations will be required before the taxonomic and phylogenetic problems in the wheats and their allies can be completely solved.

Much excellent work has been done in many countries on the cytology and genetics of the wheats, barleys, and ryes. However, there has been considerable confusion in the use of scientific plant names in papers on the taxonomy, cytology, and genetics of these cereals, and there have been great divergences of taxonomic treatments and the understanding and application of taxonomic methods. The present author considers that careful use of the International Code of Botanical Nomenclature (18) and the International Code of Nomenclature for Cultivated Plants (11) will greatly clarify the taxonomic situation in these cereals. My main objective in the present

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investigations has been to provide more precise taxonomic and nomenclatural information so that the cytologists, geneticists, and plant breeders will be in a better position to apply scientific names accurately and uniformly according to a coherent system. The present paper is a contribution towards this objective.

Methods

A series of well-defined procedures have been used for these investigations.

1. During the past two years, bibliographic reference indices have been prepared that list the species names that have been used in the genera *Triticum*, *Aegilops*, *Hordeum*, and *Secale*. More than one thousand species names have been used in these four genera. Some of these species names have now been transferred to other genera, but bibliographic work covering all of the names that have been used in these genera is necessary in order to establish the correct names for the species and species hybrids that should be recognized. Many of the original reference volumes are deposited in our library and photostatic copies of other works are being added as soon as possible. At present, about 90% of this literature has been obtained, and it is expected that most of the remainder will be added during 1959. For each species name, it is necessary to examine the original author's description to find out what he knew about the species, to establish if possible what is the type specimen, and to find out where the type or duplicates are deposited. Then studies of herbarium specimens, monographs, and floristic works show how the original author was interpreted by other botanists.

2. The second phase of the problem was to find out what monographs had been published on each genus and to determine if the taxonomic treatments were adequate. I was especially interested in determining what taxa were recognized by each monographer, the names that were used for these taxa, and what names were considered to be synonyms. Later in this paper, I shall refer to the more recent monographs that have been published on the genera *Triticum*, *Aegilops*, *Hordeum*, and *Secale*.

3. The third procedure was to grow plants of species and species hybrids in the greenhouse and experimental plots, to determine somatic chromosome numbers on representative material, and to prepare sets of voucher specimens for permanent deposit in the Herbarium of the Department of Agriculture, Ottawa (DAO), with a set of duplicates for the Herbarium of the Royal Botanic Gardens, Kew, England (K). We have facilities for the permanent storing of voucher specimens from other research workers, and urge cytogeneticists to send us specimens of the materials that are described in their papers. It is especially desirable to have specimens of each different artificial F_1 hybrid and derived amphiploid, and specimens of the parental plants. Dr. B. C. Jenkins has already deposited a fine set of some of the material of *Triticum* and *Aegilops* species and artificial amphiploids grown in the plots at The University of Manitoba, and a large number of specimens of *Hordeum* species and interspecific hybrids have been prepared from the plots of the Cereal Crops Division, Central Experimental Farm, Ottawa.

The information obtained by these procedures will enable decisions to be reached as to what species should be recognized, their correct names, and the names that are synonyms. The use of the type method is especially important in working towards an acceptable nomenclatural system. The names within each taxonomic rank are independent of those in other ranks, and the oldest legitimate epithet of each taxon is the correct one at a particular rank. This rule of priority was well understood by Zhukovsky (40), Schiemann (33), and Jakubziner (16), but remarks or treatments by some other authors show that they did not understand that one of the fundamental rules of botanical nomenclature is that the oldest legitimate epithet of each taxonomic rank must be recognized, and that the botanical nomenclature is built around these names. The correct names for a group of species are then related to the historical order of the dates of publications of the names, and the names used are not directly related to the different biological ages of species, infraspecific taxa, or hybrids.

Results

Tribe Triticeae Dumort.

The first step in the nomenclature of the wheats, barleys, and ryes is to establish the correct name of the tribe. For a long time, many authors have called it tribe Hordeae (or Hordeeae). However, Pilger (25) showed that the earliest name for the tribe, validly published as a tribe name, is Triticeae Dumort., which was published in 1823 by Dumortier (7). In his treatment, Dumortier gave a conspectus of the tribes in the family Gramineae, a Latin description of the tribe Triticeae, a conspectus of the genera, and a conspectus of the species. Under tribe Triticeae, Dumortier included some genera that would now be placed in other tribes, but he included some of the same genera listed by Pilger (25). Both Dumortier and Pilger included the genera *Triticum*, *Aegilops*, *Hordeum*, and *Secale* in the tribe Triticeae.

After the tribe Triticeae Dumort. is recognized as the earliest and correct name for the tribe, the second step is to establish the type genus of the tribe. Because of the derivation of the tribe name, the genus *Triticum* L. is automatically the type genus of the tribe Triticeae. Similarly, the typical subtribe is subtribe Triticinae which includes the genera *Triticum*, *Aegilops*, *Secale*, and others. There are several other subtribes of which subtribe Hordeinae Nevski includes the genus *Hordeum* and some other genera.

The next step is to examine the nomenclatural problems at the generic and specific levels. I shall discuss the situation in the wheats with reference to the genera *Triticum* L. and *Aegilops* L., in the barleys with reference to *Hordeum* L. sect. Hordeum, and in the ryes with reference to the genus *Secale* L.

The Taxonomy of the Wheats and Their Allies

Percival (24) discussed the ages of some of the wheats and concluded that some wheats have been under cultivation for thousands of years. Under cultivation, there have been extensive selection practices, and some of the

difficulties in studying the morphology, nomenclature, and phylogeny of the cultivated wheats are due to the production of numerous cultivars by long-term agricultural methods. We need to know what has happened in wild populations of the wheats and their relatives in nature, and what man has done with these cereals under cultivation.

Many wheat species recognized by previous authors exist only as cultivated populations, but several wheats do have wild populations. Two of these species, the diploid *T. monococcum* L. (incl. *T. boeoticum* Boiss. and *T. aegilopoides* (Link) Bal.) and the tetraploid, *T. dicoccoides* Körn. in litt. in Schweinf., have rather similar but not identical natural ranges. They occur wild in the area adjacent to the eastern end of the Mediterranean (cf. Percival (24), Hector (14), Schiemann (33), and Nevski (22)). The tetraploid *T. timopheevii* (Zhuk.) Zhuk. was described as a wild or weedy species from Western Georgia, U.S.S.R., and *T. zhukovskyi* Men. & Er., the hexaploid derived from it, was found at the same locality according to Jakubziner (16).

The annual and winter annual wheats were included in the genus *Triticum* L. by Percival (24), Flaksberger (9, 10), Hector (14), Schiemann (33), and Jakubziner (16). Three groups of wheats are generally recognized: the diploids, the allotetraploids, and the allohexaploids. Sears (36) and Lilienfeld (19) reviewed the cytology and genetics of the wild and cultivated wheats and their allies. The phylogenetic relationships have never been completely ascertained, but modern cytogenetical researches have indicated that some of the allohexaploid wheats arose from the crossing of allotetraploid wheat with *Aegilops squarrosa*. Genome AA is thought to have been derived from *Triticum monococcum* and genome DD is from *Aegilops squarrosa*. Sarkar and Stebbins (31), Sarkar (30), and Riley *et al.* (27) have suggested that genome BB has been derived from *Aegilops speltoides*. The allotetraploid wheats have the genomic formula AABB or AAGG and the allohexaploid wheats have AABBDD. At one time, the G genome was thought to have been derived from a different species (36) but it now seems more likely that the G genome is a modified B genome.

Jakubziner (16) has given the most recent classification of wheat species. He recognized three diploid species, twelve allotetraploid species, six allohexaploid species, and one auto-allohexaploid species. Several authors have recognized subspecies. Rode (28) listed some subspecies names for some of the tetraploid and hexaploid wheats (see his page 26 for subspecies). The paper by Rode (28) is usually attributed to Schübler, but the latter was chairman of Rode's examination board. Mac Key (20) understood the need for a simpler classification of the allohexaploid wheats and proposed five subspecies of *Triticum aestivum* L. To be in accord with the Botanical Code (18), subsp. *vulgare* should have been given as subsp. *aestivum* since it would be the typical subspecies. The present author agrees with Mac Key that a simpler classification of the allohexaploid wheats is needed but considers that the taxonomic treatment and nomenclature should take into account

the evidence of the hybrid origin of the cultivated polyploid wheats. Furthermore, the cultivars should be treated under article 28 of the Botanical Code (18) and the appropriate articles of the Horticultural Code (11).

Stebbins (39) listed intergeneric hybrids in the tribe TRITICEAE and discussed cytotaxonomic problems in the tribe. He considered the cytogenetic evidence and concluded that "the maintenance of *Triticum* and *Aegilops* as separate genera becomes an absurdity". Stebbins considered that all of the genera of the tribe should be united into one genus but that much more information on relationships was required before this could be done. Riley *et al.* (27) wrote "If the polyploids [the wheats] have indeed originated in the way proposed, ideas on the phylogeny of wheat must be readjusted to associate the genera *Triticum* and *Aegilops* more closely together".

I have studied this problem for some time and have reached some conclusions concerning the genera of the tribe TRITICEAE. From a practical viewpoint, it seems important to at least partly conserve generic usage in the tribe TRITICEAE as well as in other tribes in the family GRAMINEAE. Since the genera *Triticum*, *Hordeum*, and *Secale* have been recognized for such a long time, it will cause a great deal of confusion if they are reduced to subgeneric or lower rank. Changes in rank will also solve very few problems since it will still be necessary to recognize many of the same taxonomic groups, even if they are at a different rank. It is necessary to work towards a stabilized system of nomenclature that can be universally recognized. At the same time, detailed revisions of genera, subgenera, sections, and species need to be considered if they are scientifically necessary. When the taxonomic problems in the genera *Triticum* and *Aegilops* are examined, the need for further studies becomes apparent.

Zhukovsky (40) considered that *Aegilops* L. sect. SITOPSIS (Jaub. & Spach) Zhuk. was a "transitional section from the genus *Aegilops* L. to the genus *Triticum* L." and that in the genus *Triticum*, *T. monococcum* was the nearest relative of the species of sect. SITOPSIS. In *Aegilops* sect. SITOPSIS, Zhukovsky included *A. aucheri* Boiss., *A. speltoides* Tausch, *A. longissima* Schweinf. & Muschl., and *A. bicornis* (Forsk.) Jaub. & Spach. Eig (8) included five species in sect. PLATYSTACHYS Eig, and Kihara (17) listed three diploid species in sect. SITOPSIS, namely *A. speltoides* Tausch, *A. longissima* Schweinf. & Muschl., and *A. bicornis* (Forsk.) Jaub. & Spach. In sect. VERTEBRATA Zhuk., Zhukovsky (40) included *A. squarrosa* L., and Eig (8) included this species in sect. PACHYSTACHYS Eig together with *A. crassa* Boiss., *A. juvenalis* (Thell.) Eig, and *A. ventricosa* Tausch. Kihara (17) included all four species in sect. VERTEBRATA.

The system of including the parental diploid wheat species along with the allotetraploid and allohexaploid wheats of hybrid origin in the genus *Triticum* while the other parental diploid species are included in the genus *Aegilops* is nomenclaturally incorrect. The parents of hybrids or the parents of species of hybrid origin, and the hybrids or species of hybrid origin, must be included in separate taxa at the same rank. If the allotetraploid and allohexaploid

wheats are considered to have originated by intergeneric hybridization between diploid wheat and one or two diploid *Aegilops* species respectively, followed by doubling of the chromosome number of the F_1 hybrids, then the parental species and the taxa of hybrid origin have to be included in three separate genera. To satisfy this situation, diploid wheat would have to be placed in the genus *Crithodium* Link which was described by Link in 1834 in Symbolae ad floram graecam, Linnaea, vol. 9: 132–133 and taf. 3, fig. 1–5, 1834; apparently published in 1835. Link described a single species in his new genus, namely *Crithodium aegilopoides* Link. In 1842, diploid wheat was also placed in a separate genus by Seringe (37), who described a new genus, *Nivieria* Ser., which was based on *Triticum monococcum* L. (see his pages 104, 107, 111, and 114). Seringe published the combination *Nivieria monococca* (L.) Ser. If one wished to place diploid wheat in a separate genus, the generic name *Crithodium* Link would have priority. If one classified the allotetraploid and allohexaploid wheats as originating from intergeneric hybridization, they would belong in the genus ×*Triticum* L. and they would have originated from the intergeneric hybridization of *Crithodium* with *Aegilops*. When one examines this solution, it cannot be followed because there are not sufficient morphological differences to separate *Crithodium* as a genus from certain species of *Aegilops*, particularly *A. bicornis* and *A. speltoides* of sect. SITOPSIS.

The morphological differences between *Triticum monococcum* and the species of *Aegilops* sect. SITOPSIS are adequate only to separate the taxa at the specific level, and in my opinion the differences are not even satisfactory to clearly establish distinct sections. Compared with the species of sect. SITOPSIS, *Triticum monococcum* has broader, coarser spikes, spikelets usually crowded and imbricate in the lateral rows, and each glume bicarinate (two raised carinal veins) with the adaxial vein being sharply winged and the abaxial vein being merely rugose (i.e. raised on the surface). *Aegilops bicornis* and *A. speltoides*, of sect. SITOPSIS, have more delicate, narrower spikes, spikelets usually more remotely spaced in each lateral row, and each glume unicarinate, with a raised prominent carinal adaxial vein (winged slightly towards the apex) and a raised lateral abaxial vein (several lateral veins in *speltoides*).

I now propose a solution that establishes a basis for a correct nomenclature and takes into account the biological situation in the diploid, allotetraploid, and allohexaploid wheats, and the diploid species, allopolyploid species of hybrid origin, and other natural and artificial interspecific hybrids of *Aegilops* and hybrids of *Triticum* × *Aegilops*. Under the proposed treatment, there are interspecific hybrids and species of interspecific hybrid origin within an enlarged genus *Triticum* L. emend. which includes both the series of wheats and the diploid and allopolyploid *Aegilops* species.

Since *Triticum* L. is the type of the tribe TRITICEAE, I am accepting *Triticum* L. as the name for the genus to include both the annual and winter annual wheats (*Triticum* L.) and the species placed in the genus *Aegilops* L. by Zhukovsky (40), Eig (8), and Kihara (17). By lumping these two genera

into one genus, a simple and effective nomenclature can be established. The idea of lumping these two genera is not new and partial treatments have been made long ago by several authors, for example, Grenier and Godron (13) and Richter (26).

Taxonomic Treatment

Genus *Triticum* L., nom. emend., Gen. Pl., ed. 5: 37. 1754; and Sp. Pl., ed. 1, vol. 1: 85–86. 1753. Triticis annuis inclusis, perennis exclusis, sed Aegilopsis speciebus inclusis. Syn.: *Aegilops* L., Gen. Pl., ed. 5: 470. 1754. and Sp. Pl., ed. 1, vol. 2: 1050–1051. 1753. *Crithodium* Link, Linnaea 9: 132–133 and taf. 3, 1834, published 1835. *Nivieria* Ser., Ann. Soc. Roy. Lyon, vol. 5: pp. 104; 107; 111 and 114. 1842.

The lectotype species (hybrid) of the genus *Triticum* L. emend. is *T.* × *aestivum* L. (pro sp.), Sp. Pl., ed. 1, vol. 1: 85. 1753. It was selected as type by Hitchcock (15).

Description of the genus *Triticum* L. emend.: Plants tufted, annual or winter annual; spikes bearded or beardless, cylindric or compressed, with the spikelets in two lateral rows and one spikelet per node; glumes with one or two keels or without keels, with a variable number of slightly to definitely raised veins and usually one or two more prominent veins that are associated with the keels, with the glume teeth, or with the awns, when these are present; glume apices bifid, biaristate, triaristate, multiaristate, or truncate with one vein excurrent.

Flaksberger (9) grouped the diploid, allotetraploid, and allohexaploid wheats in three sections under *Triticum* L. Zhukovsky (40), Eig (8), and Kihara (17) classified the species of *Aegilops* L. in numerous sections, and each author had a different system of grouping. I regard the genus *Triticum* L., as I have emended it, to include all of the diploid species, allopolyploid species (hybrid origin), and interspecific hybrids that were included in *Triticum* L. and *Aegilops* L. or in *Triticum* L. × *Aegilops* L. by these authors.

I consider it inadvisable to classify these species and species of hybrid origin in sections. The diploid species should not be classified in the same sections as the allopolyploid species of interspecific hybrid origin. This point was already discussed by Lilienfeld (19). If the 10 diploid species are classified at the same level as the sections in the monographs of Flaksberger (9), Zhukovsky (40), and Eig (8), seven sections would be required for the 10 diploid species. Four sections would be unispecific and three sections would be bispecific. These sections are too small to be useful. As for the allotetraploid species of interspecific hybrid origin, only those species that originated from the crossing of diploid species in the same two parental sections could be classified in the same section if sectional classification were used. It would require a large number of sections to classify all of the natural and artificial interspecific hybrids and species of hybrid origin. These sections would be unispecific, bispecific, or quadrispecific. All of the needed sections that would contain diploids, allopolyploids, and artificial and natural hybrids comprise the genus *Triticum* L. emend.

For the reasons given above, I am not using a complicated sectional classification but treat the diploid species under I and then consider the allopolyploids under II and other interspecific hybrids under III. Kihara (17) has given genomic formulae for the species of *Aegilops* L. and Sears (36) and Lilienfeld (19) have given the genomic formulae for some of the wheats.

The infrageneric taxa of *Triticum* L. emend. are:

I. Diploid Species

Triticum monococcum (genome AA); *T. bicorne; T. speltoides* (possibly genome BB); *T. comosum; T. uniaristatum; T. longissimum; T. umbellulatum; T. tripsacoides; T. dischasians;* and *T. aegilops* (genome DD).

II. Allopolyploids (Hybrid Origin)

(*a*) Allotetraploid wheats: *Triticum turgidum* L. emend. (genome AABB or AAGG). One auto-allohexaploid form belongs here.

(*b*) Allohexaploid wheats: *Triticum* × *aestivum* L. emend. (genome AABB-DD).

(*c*) Allopolyploid species of interspecific hybrid origin: *Triticum ovatum; T. triaristatum; T. kotschyi; T. triunciale; T. cylindricum; T. macrochaetum; T. crassum; T. turcomanicum; T. juvenale;* and *T. ventricosum.*

III. Other Artificial and Natural Interspecific Hybrids

Treatment of the Infrageneric Taxa of *Triticum* L. emend.

I. Diploid Species (2n = 14)

1. *Triticum monococcum* L., Sp. Pl., ed. 1, vol. 1: 86. 1753. Lectotype specimen: Savage (32) Catalogue No. 104.4 in the Linnaean Herbarium, London (LINN; photograph, DAO!). Genomic formula: AA.

Description of T. monococcum L.—Spikes long-bearded, compressed, narrow across the faces, much broader across the two-rowed profiles; spikelets compressed, usually crowded and imbricate in the lateral rows; each glume bicarinate (two raised carinal veins) with the adaxial vein being sharply winged and the abaxial vein being merely rugose (i.e. raised on the surface); unequally two-toothed; lemmas long-awned.

The geographical range of *T. monococcum* L. is Greece, Bulgaria, and the Crimea to the Trans-Caucasus region in the southwestern part of the U.S.S.R. and Asia Minor, Palestine, Syria, and Iraq (see Hector (14), Nevski (22), Percival (24), and Schiemann (33)).

Many species names have been used for the diploid wheats with homogenomic formula AA, but the evidence indicates that they belong to a single species, *T. monococcum* L. Artificial autotetraploids (AAAA) also belong here. Flaksberger (9) placed the diploid wheats in sect. Monococca Flaksb.

Some authors have used *T. boeoticum* Boiss, *T. thaoudar* Reut. in Bourg. ex Hausskn., or the illegitimate name, *T. aegilopoides* (Link) Bal., for the wild diploid wheat species, and *T. monococcum* L. as the name for the cultivated diploid wheats. Article 28 of the Botanical Code (18) indicates that the wild and cultivated plants belong to the same species, and that plants

that have undergone change by selection or other processes under cultivation should be named as cultivars under the Horticultural Code (11). Schiemann (33) and Jakubziner (16) agreed that *T. aegilopoides* (Link) Bal. is an illegitimate name because of the earlier homonym, *T. aegilopoides* Forsk.

To fully clarify the taxonomy of the diploid wheats, it will be necessary to examine the morphology of the type specimen of *T. monococcum* L. Then a study of specimens of diploid wheat from the whole natural range of the species should show how many infraspecific taxa should be recognized. The cultivars of diploid wheat should be named under the Horticultural Code (11) but it should be possible to determine at least some of the relationships between the wild plants and the cultivars.

2. *Triticum bicorne* Forsk., Descript. Plant. Fl. Aegypt.-Arab. 1: 26. 1775. Syn.: *Aegilops bicornis* (Forsk.) Jaub. & Spach., Illustr. Pl. Orient. 4: 10–11 and tab. 309. 1850–53.

3. *Triticum speltoides* (Tausch) Gren. ex Richter, Plant. Europ. 1: 129. 1890. Based on *Aegilops speltoides* Tausch, Flora 20: 108–109. 1837. *T. speltoides* (Tausch) Gren., Fl. Massil. Adv. in Mem. Soc. Emul. Doubs, ser. 3, vol. 2: 434. 1857, published 1858. *Nomen provisorium.*

Sarkar and Stebbins (31), Sarkar (30), and Riley *et al.* (27) thought that the BB genome of the tetraploid wheats may have come from this species, but the hypothesis has not been completely proved. *Triticum speltoides* has a natural range from Palestine to Turkey.

There are two forms in *Triticum speltoides.* About one-third of our specimens of this species have coarsely awned terminal spikelets with all the lateral spikelets awnless. The other two-thirds of our specimens have coarsely awned terminal spikelets with long-awned lemmas in the lateral spikelets. The names for these two forms are:

(*a*) *Triticum speltoides* (Tausch) Gren. ex Richter f. *speltoides*, l.c., is the typical form. When Tausch described *speltoides*, he wrote "spica subulata, glumis calycinis omnibus muticis truncatis obsolete 1-dentatis, flosculis muticis, spiculae terminalis aristatis". This description accurately describes the one-third group of specimens with awns only in the terminal spikelets. Eig (8) interpreted the typification of this species correctly, and he included f. *speltoides* under *Aegilops speltoides.* Zhukovsky (40) used the name *Aegilops aucheri* Boiss. for this taxon.

(*b*) Zhukovsky (40) applied the name *Aegilops speltoides* to the many-awned form and Eig (8) used the name *Aegilops ligustica* (Savign.) Cosson. The name for the form with long-awned lemmas in the lateral spikelets is:

Triticum speltoides (Tausch) Gren. ex Richter f. **ligusticum** (Savign.) Bowden, comb. nov., based on *Agropyrum ligusticum* Savign., Atti della Ottava Riunione scienziati Italiani, Genova, 601–602. 1846, published 1847. See *Agropyrum ligusticum* Savignone, Flora, vol. 30: 568–569. 1847. The awns in the terminal spikelets of specimens of *ligusticum* are often somewhat coarser than the awns in the lateral spikelets. A few specimens are intermediate between f. *speltoides* and f. *ligusticum*, that is, in these intermediates there are a few awned lemmas in the lateral spikelets.

4. *Triticum comosum* (Sibth. & Sm.) Richter, Plant. Europ. 1: 128. 1890. Based on *Aegilops comosa* Sibth. & Sm., Fl. Gr. 1: 75 and tab. 94. 1806. See also Fl. Gr. Prodr. 1: 72. 1806.

5. *Triticum uniaristatum* (Vis.) Richter, Plant Europ. 1: 128. 1890. Based on *Aegilops uniaristata* Vis., Fl. Dalm. 3: 345. 1852.

6. *Triticum* **longissimum** (Schweinf. & Muschl. in Muschl.) Bowden, comb. nov., based on *Aegilops longissima* Schweinf. & Muschl. in Muschl., A Manual Flora of Egypt, 1: 156. 1912.

7. *Triticum* **umbellulatum** (Zhuk.) Bowden, comb. nov., based on *Aegilops umbellulata* Zhuk., Bull. Appl. Bot. Gen. Pl. Br. 18: 447–448 and 483–489. 1928.

8. *Triticum* **tripsacoides** (Jaub. & Spach) Bowden, comb. nov., based on *Aegilops tripsacoides* Jaub. & Spach, Illustr. Pl. Orient., 2: 121 and tab. 200. 1846. Syn.: *A. mutica* Boiss., Diagn. Plant. Orient. Nov., ser. 1, fasc. 5: 73–74. 1844. *T. muticum* (Boiss.) Hackel in Fraser, Ann. Scott. Nat. Hist., p. 103. 1907. Non *T. muticum* Rode, Charact. et Descr. Cereal. in Hort. Tubing. pp. 10–11. 1818.

There are two forms in *Triticum tripsacoides*. The first form has glumes that are conspicuously white-hirsute and the second form has glabrous glumes. Zhukovsky (40) included the hirsute glume form under *Aegilops mutica* Boiss. ssp. *tripsacoides* (Jaub. & Spach) Zhuk. and Eig (8) listed it under *Aegilops mutica* Boiss. var. *typica* Eig. Zhukovsky (40) included the glabrous-glume form under *Aegilops mutica* Boiss. ssp. *loliacea* (Jaub. & Spach) Zhuk. and Eig (8) listed it under *Aegilops mutica* Boiss. var. *loliacea* Eig. The names for these two forms are:

(*a*) *Triticum tripsacoides* (Jaub. & Spach) Bowden f. *tripsacoides*, l.c., is the typical form with conspicuously white-hirsute glumes. When Jaubert and Spach originally described this species, the main part of their description referred to the hirsute glume form. They also referred to "β: spiculis glabris". In Tabula 200 of their work, they showed both forms. In Fig. A and Fig. 2, they showed the "forme à épillets hispides" which is f. *tripsacoides*. In Fig. B and Fig. 10, they showed the "forme à épillets glabres". The type specimen is "In Phrygia, prope Thermas hieropolitanas (hodie *Pambouk Calessi*), legit Jaubert, junio 1839".

(*b*) *Triticum tripsacoides* (Jaub. & Spach) Bowden f. **loliaceum** (Jaub. & Spach) Bowden, comb. nov., based on *Aegilops loliacea* Jaub. & Spach, Illustr. Pl. Orient., 4: 23 and tab. 317. 1850–53. Forma *loliacea* has glabrous glumes. When Jaubert and Spach described *Aegilops loliacea*, they included both the glabrous and hirsute glume forms. In their tabula 317, the drawing of the two spikes shows the glabrous glume form and Fig. 4 shows a glabrous glume. Figure 3 shows a glume of the hirsute glume form (f. *tripsacoides*). The monographic treatments of Zhukovsky (40) and Eig (8) restricted the name *loliaceum* to the glabrous glume form. The type specimen is "Cappadocia, ad Euphratem, legit Aucher-Eloy! (Plant. exs. no. 2977, in Herb. Mus. Par.)". This is an isotype of *Aegilops mutica* Boiss. of which the holotype is

"Hab. in Cappadociâ ad Euphratem Aucher No. 2977", Herb. Geneva (G); photograph, DAO! Boissier's description of *Aegilops mutica* referred only to the hirsute glume form (f. *tripsacoides*).

9. *Triticum* **dichasians** (Zhuk.) Bowden, comb. nov., based on *Aegilops caudata* L. ssp. *dichasians* Zhuk., Bull. Appl. Bot. Gen. Pl. Br. 18: 512 and figs. 42 and 45. 1928. Syn.: *Aegilops caudata* L., Sp. Pl., ed. 1, vol. 2: 1051. 1753. *T. caudatum* (L.) Godr. & Gren. in Gren. & Godr., Fl. Fr. 3: 603. 1856. Non *T. caudatum* Pers., Syn. 1: 110. 1805.

Eig (8) considered that the specimen labelled *caudata* in the Linnaean Herbarium was not a specimen of this species. The sheet of Savage (32) Catalogue No. 1218.5 in the Linnaean Herbarium, London (LINN; photograph, DAO!), was labelled "2 caudata" by Linnaeus but the right-hand specimen appears to me to belong to another species (see under *T. aegilops*) and I regard it as a misidentified specimen. In the original description of *A. caudata* L., Linnaeus wrote:

"*Habitat in* Creta.

Glumae omnes, excepta ultima, bidentatae sunt".

The geographical area and the description of the bidentate glumes fit the species called *A. caudata* L. by Zhukovsky (40) and Eig (8). The right-hand specimen of 1218.5 cannot be selected as type of this species because it does not fit the original description of Linnaeus.

10. *Triticum aegilops* P. Beauv. ex R. & S., Syst. Veg. 2: 769–770. 1817. Pro parte, typo Linnaei incluso. Based on *Aegilops squarrosa* L., Sp. Pl., ed. 1, vol. 2: 1051. 1753. Non *T. squarrosum* Roth, Neue Beytr. zur Bot. 1: 128. 1802. Syn.: *T. aegylops* P. Beauv., *nomen nudum*, Essai Nouv. Agr., Nouv. Genr. Graminees, Index p. 180. 1812. *T. squarrosum* (L.) Raspail, Ann. Soc. Nat. ser. 1 (5): 435. 1825. *A. squarrosa* sensu Willd., Sp. Pl. 4: 944. 1805. Pro parte (syn. Linn. incluso). *A. squarrosa* sensu Schreber, Beschr. Gräser, vol. 2: 44–45 and tab. 27, fig. 2. 1772 (–1779). *A. squarrosa* sensu Tausch, Flora 20: 108. 1837. *A. tauschii* Cosson, Not. Quelq. Pl. 2: 69. 1849. *T. tauschii* (Coss.) Schmalh., Fl. Centr. & S. Russia 2: 662. 1897. Non *A. squarrosa* sensu Cosson, Not. Quelq. Pl. 2: 68-69. 1849.

The genomic formula of *T. aegilops* is DD according to Kihara (17).

When Linnaeus published the name *A. squarrosa* L., he wrote:

"Aegilops spica subulata aristis longiore.

Habitat in Oriente. *Tournefort*".

The words "*Habitat in* Oriente" restrict the application of the epithet *squarrosa* to the species from Central Asia. In 1837, Tausch recognized that botanists were including three species under the name *A. squarrosa*; he redescribed *A. squarrosa* and described two additional species, *A. ventricosa* Tausch and *A. speltoides* Tausch. Tausch gave some of the pertinent morphological characteristics of the Central Asiatic species, *A. squarrosa*, and gave the geographical range as Iberia (Georgia, U.S.S.R.) and Tauria.

The type specimen of *A. squarrosa* L. has not been designated. Presumably it should be a specimen of the Central Asiatic species in the Linnaean

Herbarium or the Tournefort specimen of this species. In the Linnaean Herbarium, London, the sheet of Savage (32) No. 1218.9 (LINN; photograph, DAO!) is labelled "3 squarrosa" in Linnaeus' handwriting but the specimen is not satisfactory as type since it appears to be a specimen of *T. triunciale* (L.) Raspail. I regard it as a misidentified specimen.

Two of the three specimens of the two sheets labelled "caudata" in the Linnaean Herbarium, London, namely Savage (32) Catalogue No. 1218.5 and 1218.6 (LINN; photographs, DAO!), appear to be the Central Asiatic species and not *A. caudata* L. from the Eastern Mediterranean and adjacent areas. I regard these two sheets as misidentified specimens.

The geographical range of the Central Asiatic species *T. aegilops* (genome DD) is from the Caucasus region, U.S.S.R. to Tien Shan and from Iran to Afghanistan (Nevski, 22; Eig, 8; Zhukovsky, 40; and Kihara, 17).

II. Allopolyploids (Hybrid Origin)

Cytogenetic evidence indicates that the allotetraploids and allohexaploids originated by interspecific hybridization of the diploids followed by doubling of the chromosome number.

II (a). Allotetraploid Wheats with Genome AABB or Genome AAGG (2n = 28) and a Related Auto-allohexaploid Form (2n = 42)

Jakubziner (16) recognized twelve species of allotetraploid wheats: *T. turgidum* L.; *T. polonicum* L.; *T. dicoccon* Schrank; *T. durum* Desf.; *T. dicoccoides* Körn. in litt. in Schweinf.; *T. carthlicum* Nevski (= *T. persicum* Vav. ex Zhuk.); *T. palaeocolchicum* Men.; *T. turanicum* Jakubz. (= *T. dicoccoides* Körn. race *T. orientale* Perc.); *T. aethiopicum* Jakubz. (= *T. abyssinicum* (Vav.) Flaksb.); *T. timopheevii* (Zhuk.) Zhuk.; *T. araraticum* Jakubz. (= *T. armeniacum* (Jakubz). Mukush., non *T. armeniacum* (Stolet.) Nevski); and a new species from Iran. Jakubziner (16) thought that the auto-allohexaploid, *T. zhukovskyi* Men. & Er., was closely related to *T. timopheevii*. Sears (36) gave the genomic formula AABB for the first six species and AAGG formula for *T. timopheevii* and *T. araraticum*.

Of the 12 allotetraploid species recognized by Jakubziner (16), eight species are apparently known only as cultivated plants, namely, *T. turgidum*, *T. polonicum*, *T. dicoccon*, *T. durum*, *T. carthlicum*, *T. palaeocolchicum*, *T. turanicum*, and *T. aethiopicum*. The other species recognized by Jakubziner are apparently known at least in part as wild populations. The largest range for a wild allotetraploid wheat is that of *T. dicoccoides*, which occurs in the area east of the eastern end of the Mediterranean. *Triticum timopheevii* was found in Georgia, U.S.S.R. and was considered by Nevski (22) to be endemic there. *Triticum zhukovskyi* was found in the same area. *Triticum araraticum* was described from the Trans-Caucasus.

Flaksberger (9) included some of these allotetraploid wheats in sect. DICOCCOIDEA Flaksb. The allotetraploid wheats with heterogenomic formula AABB are thought to have originated from hybridization followed by doubling of the chromosome number, involving crosses between *T. monococcum* and one of the other diploid species which is probably *T. speltoides* (given as

A. speltoides) according to Sarkar and Stebbins (31), Sarkar (30), and Riley *et al.* (27). If the geographical ranges of the diploid *T. monococcum*, the diploid *T. speltoides*, and the other diploid species of *Triticum* that are native in the area east of the eastern end of the Mediterranean are compared with the geographical ranges of the wild allotetraploid wheats such as *T. dicoccoides*, *T. timopheevii* and *T. araraticum*, it is apparent that if *T. speltoides* is the source of the BB genome of *T. dicoccoides*, then *T. speltoides* is also the source of the BB genome or the GG genome in the other allotetraploid wheats. *Triticum speltoides* (probably genome BB) occurs in a relatively small area from Palestine to Turkey. The three wild allotetraploid wheats mentioned above occupy a relatively larger area from Palestine and Syria to Iran and Georgia, U.S.S.R. *Triticum dicoccoides* has apparently spread from the original center of development and the wild populations eventually spread over a relatively large area.

I consider that *T. dicoccoides*, *T. timopheevii*, *T. zhukovskyi*, and *T. araraticum* belong to the original wild populations, and that the remainder of the allotetraploid wheats have been brought into cultivation from some of these populations. The cultivars have undergone selection by man over a long period of time.

I propose that all of these allotetraploid wheats should be regarded as belonging to a single species of hybrid origin, which also includes the autoallohexaploid *T. zhukovskyi*, which is closely related to *T. timopheevii*. This species complex (AABB and AAGG) is of hybrid origin presumably from the crossing of *T. monococcum* (genome AA) with *T. speltoides* (genome BB) which formed sterile F_1 hybrids that gave rise to fertile populations following doubling of the chromosome number. If the allotetraploid wheats (AABB) had failed to establish a natural range in nature, or were very localized in their natural distribution, they would be regarded as belonging to an interspecific hybrid complex. But, since the wild populations of allotetraploid wheats have a definite and extensive geographical range in the wild, this species complex (AABB) cannot be treated as an interspecific hybrid. It must be regarded as a species of hybrid origin.

The oldest specific name of any of the allotetraploid wheats is *Triticum turgidum* L. I shall emend this name to apply to all of the wheats in the AABB and AAGG complex. The four taxa that occur as wild plants will be treated according to the Botanical Code (18) and the wheats that are known only as cultivated plants will be treated as cultivars according to article 28 of the Botanical Code (18) and the appropriate articles, particularly article 13 of the Horticultural Code (11). The name for this species is:

Triticum turgidum L., nom. emend., Sp. Pl., ed. 1, vol. 1: 86. 1753. Lectotype specimen: Savage (32) Catalogue No. 104.2 in the Linnaean Herbarium, London (LINN; photograph, DAO!).

The description of *T. turgidum* L. emend. is: Spikes usually bearded and compressed, narrow across the faces, much broader across the two-rowed profiles; spikelets usually compressed, crowded, imbricate in the lateral rows;

each glume unicarinate, the carina being formed by the sharply winged adaxial vein, and the other main abaxial lateral vein quite conspicuous, but usually not definitely rugose; lemmas usually long-awned.

The infraspecific taxa of *Triticum turgidum* L. emend. are:

1. Groups of cultivars:
   - (*a*) turgidum
   - (*b*) polonicum
   - (*c*) dicoccon
   - (*d*) durum
   - (*e*) carthlicum
   - (*f*) palaeocolchicum
   - (*g*) turanicum
   - (*h*) aethiopicum
2. var. *dicoccoides*
3. var. *timopheevii*
   - (*a*) f. *timopheevii*
   - (*b*) f. *zhukovskyi*
4. var. *tumanianii*

1. The Groups of cultivars of *Triticum turgidum* L. emend.i nclude all of the cultivated allotetraploid wheats with genome AABB. The following eight groups are recognized:

1 (*a*) Group turgidum, l.c., the typical group, i.e., based on *T. turgidum* L., Sp. Pl. ed. 1, vol. 1: 86, 1753. The lectotype specimen is cited above. Many cultivars belong under this group, for example the varieties listed on pages 247–248 of Percival (24).

1 (*b*) Group polonicum. Based on *T. polonicum* L., Sp. Pl., ed. 2, vol. 1: 127. 1762. Lectotype specimen: Savage (32) Catalogue No. 104.3 in the Linnaean Herbarium, London (LINN; photograph, DAO!). Percival (24) has listed many cultivars that belong in this group, on pages 233–234 of his monograph.

1 (*c*) Group dicoccon. Based on *T. dicoccon* Schrank, Baier. Fl. 1: 389. 1789. Percival (24) listed numerous varieties on pages 192–193 that belong in group dicoccon as cultivars.

The name *Triticum dicoccon* Schrank was originally published in a somewhat ambiguous manner and various authors have cited the original name either as a species or as a variety of *T. spelta* L. In the original publication of Schrank, the name *dicoccon* was prefixed with an asterisk instead of a number as for most of the species in the work. Some authors have taken this asterisk to mean that *dicoccon* was intended as a variety of *T. spelta* which immediately precedes it. The index of the work is headed "Verzeichniss der Gattungen und Arten" and it includes all of the numbered species and the names prefixed with asterisks. Other internal evidence strongly indicates that *T. dicoccon* was meant to be the name of a species. Although Schrank originally used the spelling "dicoccon", most other authors have spelled it "*dicoccum*". I am restoring the original spelling of *dicoccon* as used by Schrank.

1 (*d*) Group durum. Based on *T. durum* Desf., Fl. Atlant. 1: 114. 1798. Type specimen: In Desfontaines' Herbarium (P; photograph, DAO!). Syn.: *T. dicoccoides* Körn. race *T. pyramidale* Perc., The Wheat Plant, pp. 156–157, 262–264 and figs. 161; 162; and 163. 1921. Percival (24) listed numerous varieties that belong under group durum as cultivars (his pages 211–212).

1 (*e*) Group carthlicum. Based on *T. carthlicum* Nevski in Komarov's Flora U.S.S.R., 2: 685–686. 1934. Syn.: *T. persicum* Vav. ex Zhuk., Bull. Appl. Bot. Pl. Br. 13 (1): 46–47 and tab. 9–12. 1923. *T. persicum* Vav., Ann. Acad. Agron. Petrov., 1–4: 177–178. 1919. *Nomen nudum.* Non *T. persicum* (Boiss.) Aitch. & Hemsl., Trans. Linn. Soc., ser. 2, vol. 3: 127. 1888.

Nevski (22) stated that group carthlicum was cultivated in Eastern and Southern Trans-Caucasus. Zhukovsky (41) discussed group carthlicum (Under *T. persicum*) and stated that it was cultivated in Georgia, Daghestan and Armenia, U.S.S.R., and Eastern Turkey. Zhukovsky wrote "It appeared that this was an old aboriginal culture from mountainous Georgia".

1 (*f*) Group palaeocolchicum. Based on *T. palaeo-colchicum* Men., Commun. Georgian Br. Acad. Sci., U.S.S.R. (Mitteil. Georgischen Abt. Akad. Wiss.), vol. 1, no. 9: 686–687. 1940. Syn.: *T. dicoccum* Schrank var. *chvamlicum* Supat., Bull. Inst. Exper. Agr. Georgia, 1: 92; 96 and 98 (diagnosis) and plates 2 and 3. 1929. *T. palaeo-colchicum* Men. var. *chvamlicum* (Supat.) Men. and var. *rubidum* Men., Commun. Georgian Br. Acad. Sci., U.S.S.R., vol. 1, no. 9: 686. 1940. Jakubziner (16) stated that palaeocolchicum was originally described from Western Georgia.

1 (*g*) Group turanicum. Based on *T. turanicum* Jakubz., Selektzia i semenovodstvo, no. 5 (163): 46. 1947. Syn.: *T. dicoccoides* Körn. race *T. orientale* Perc., The Wheat Plant, pp. 155, 204–205 and fig. 136. 1921. Non *T. orientale* M. Bieb., Flor. Taur.-Cauc. 1: 86. 1809.

1 (*h*) Group aethiopicum. Based on *T. aethiopicum* Jakubz., Selektzia i semenovodstov, no. 5 (163): 46. 1947. Syn.: *T. abyssinicum* (Vav.) Flaksb., Key to the True Cereal Crops, 4th ed. p. 92. 1939. Non *T. abyssinicum* Steud., Syn. Pl. Gram.: 342. 1855.

Hybrids involving crosses between any of these eight groups of cultivars should be classified as hybrid cultivars under *Triticum turgidum* L. emend. They will be intraspecific hybrids under the new classification rather than interspecific hybrids under older classifications.

2. *Triticum turgidum* L. emend. var. **dicoccoides** (Körn. in litt. in Schweinf.) Bowden, comb. nov., based on *T. dicoccoides* Körn. in litt. in Schweinf., Bericht. Deutsch. Bot. Ges., 26a: 309–322. 1908. Syn.: *T vulgare* Vill. var. *dicoccoides* Körn., Verh. Naturhist. Ver. Jahr. 46, Sitzgsber. Niederrhein Ges. f. Natur.-Heilj., Sitzung vom 11. Marz 1889. p. 21. 1889. *Nomen nudum. T. dicoccoides* Körn. subsp. *palestinicum* Jakubz., Bull. Appl. Bot. Gen. Pl. Br., ser. 5, No. 1: 155, 157, 161, and 195. 1932.

Triticum turgidum var. *dicoccoides* ranges from Syria and Palestine to Western Iran (Hector (14), Nevski (22), Percival (24), and Schiemann (33)).

3. *Triticum turgidum* L. emend. var. **timopheevii** (Zhuk.) Bowden, comb. nov., based on *T. dicoccum* Schrank var. *Timopheevi* Zhuk., Scient. Papers Appl. Sect. Tiflis Bot. Gard., 3: pp. 1–3 and fig. 1–4. 1924. Syn.: *T. Timopheevi* (Zhuk.) Zhuk., Bull. Appl. Bot. Gen. Pl. Br., 19, No. 2: 64. 1928. *Triticum dicoccum dicoccoides* Körn. var. *Timopheevi* Zhuk., Bull. Appl. Bot. Gen. Pl. Br., 19, No. 2: 64. 1928. *Nomen ex syn.*

3 (*a*) f. *timopheevii*, the typical form, l.c. In the description of *timopheevii* published by Zhukovsky in 1928, he considered it to be a "weed species, seldom in wild state, peculiar only to Georgia". In 1944, Zhukovsky (41) stated that *timopheevii* was "inherent to Western Georgia" and that it was "difficult for thrashing and its ears are brittle". Nevski (22) considered that *timopheevii* was endemic in Eastern Trans-Caucasus.

3 (*b*) f. **zhukovskyi** (Men. & Er.) Bowden, comb. nov., based on *T. zhukovskyi* Men. & Er., Comm. Georgian Br. Acad. Sci., U.S.S.R., No. 16: p. ? 1958. (*Non vide.*) According to Jakubziner (16), the auto-allohexaploid f. *zhukovskyi* was found at the same locality in Western Georgia, U.S.S.R. as the allotetraploid f. *timopheevii*, and the two forms are closely related. The genomic formula of f. *zhukovskyi* appears to be AAAGGG.

4. *Triticum turgidum* L. emend. var. **tumanianii** (Jakubz.) Bowden, comb. nov., based on *T. dicoccoides* Körn. subsp. *armeniacum* Jakubz. var. *Tumaniani* Jakubz., Bull. Appl. Bot. Gen. Pl. Br., ser. 5, No. 1: 155, 156, 159, 163, and 197. 1932. Syn: *T. dicoccoides* Körn. subsp. *armeniacum* Jakubz. var. *Nachitchevanicum* Jakubz., Bull. Appl. Bot. Gen. Pl. Br., ser. 5, No. 1: 163; 197. 1932. *T. araraticum* Jakubz., Selektzia i semenovodstvo, No. 5 (163): 46. 1947. *T. armeniacum* (Jakubz.) Mukushina, Compt. Rend. (Doklady) Acad. Sci., U.S.S.R., n.s. 21: 345–348. 1938. Non *T. armeniacum* (Stolet.) Nevski, in Komarov's Flora U.S.S.R., vol. 2: 683. 1934.

In the original description of var. *tumanianii*, Jakubziner stated that most specimens had white spikes and awns, and that the plants with red spikes and awns were of rarer occurrence. Jakubziner's descriptions of the localities where he collected var. *tumanianii* as well as the descriptions of the morphology of the specimens, supported his view that these were wild allotetraploid wheats. Variety *tumanianii* occurs in the Trans-Caucasus region, in both the Armenian S.S.R. and Azerbaijan (= Nakhichevan) S.S.R.

II (*b*). *Allohexaploid Wheats with Genomic Formula AABBDD* (*2n* = *42*)

All of the allohexaploid wheats with AABBDD genome have been derived from crosses between the allotetraploid wheats, *T. turgidum* L. emend. (genome AABB) and *T. aegilops* P. Beauv. ex R. & S. (= *Aegilops squarrosa* L.) (genome DD). Presumably the sterile F_1 hybrids (ABD) gave rise to fertile populations following the doubling of the chromosome number to form allohexaploids (AABBDD). All of these AABBDD allohexaploids exist at present as cultivated wheats and they do not have a natural geographical range in the wild, similar, for example, to the range of the wild allotetraploid wheat, *T. turgidum* var. *dicoccoides*.

The original AABBDD allohexaploid hybrids could have been formed in three ways. First, the hybrids could have been developed in fields where cultivated allotetraploid wheat (genome AABB) grew together with the diploid species *T. aegilops* (= *A. squarrosa*) in the area where *T. aegilops* is native in Central Asia (see Fig. 8 of Kihara (17)). When the hybrids were first formed, they would occur as weeds in the fields of allotetraploid wheat. Second, cultivated allotetraploid wheat could have crossed with *T. aegilops*

that grew in uncultivated areas adjacent to the cultivated fields. Allohexaploid wheats could have developed as populations in the uncultivated areas. Third, hybrids could have developed in the wild state from the crossing of the wild allotetraploid, *T. turgidum* var. *dicoccoides*, and the diploid *T. aegilops*, or from the crossing of other wild allotetraploid wheats and *T. aegilops*. The area where this could occur in nature is the area where the range of *T. aegilops* overlaps the ranges of *T. turgidum* var. *dicoccoides* and the other wild allotetraploid wheats, i.e. in the Trans-Caucasus region and in Northern Iran.

The conspicuous differences between some of the allotetraploid and allohexaploid wheats would draw attention to the allohexaploids, and these could then have been selected for growing as separate crops. It is well known that many of the cultivated crops in parts of Central Asia and adjacent areas are mixtures which would reflect the original weedy nature of some of the constituent elements. The primitive allohexaploid wheats have by now undergone much selection under cultivation and more recently by planned breeding programs. Many named cultivars are now available.

The hybrid origin of the AABBDD allohexaploids is no longer a tentative hypothesis since a great deal of evidence has now accumulated to support the concept (Kihara (17), Lilienfeld (19), and Sears (36)). The AABBDD allohexaploids cannot be considered to belong to a species (of hybrid origin) because a species would have established a definite geographical range in nature, similar, for example, to that of *T. turgidum* var. *dicoccoides* which belongs to a species of hybrid origin. None of the AABBDD allohexaploid wheats have such a comparable geographical area in the wild. If wild populations of AABBDD allohexaploids do occur, they must have a very localized distribution. For this reason, I regard the AABBDD allohexaploids as belonging to a hybrid complex, i.e. an interspecific hybrid or "species hybrid". I consider that all of these AABBDD allohexaploids belong to a single collective interspecific hybrid, and I shall treat this collective taxon according to the appropriate articles, especially appendix I of the Botanical Code (18) and the articles of the Horticultural Code (11). Examples of interspecific hybrids that have been given binary names are *Verbascum* × *schiedeanum* Koch and *Primula* × *kewensis* W. Wats.

Jakubziner (16) recognized six wheats in this species hybrid complex: *T. aestivum* L.; *T. spelta* L.; *T. compactum* Host; *T. sphaerococcum* Perc. (published by Percival (24) as *T. dicoccoides* Körn. race *T. sphaerococcum* Perc.); *T. macha* Dek. & Men.; and *T. vavilovii* Jakubz. Sears (36) gave the genomic formula AABBDD for these six species. All six of these wheats are cultivated and I propose that they should be treated as groups of cultivars under a collective species name according to article 28 and Appendix I of the Botanical Code (18) and article 13 of the Horticultural Code (11). The numerous varieties of *T. aestivum L.* listed by Mansfeld (21) belong here as cultivars.

One of the oldest specific epithets and the type of the genus *Triticum* L. is the name for this collective interspecific hybrid: *Triticum* × *aestivum* L.

(pro sp.), nom emend., Sp. Pl., ed. 1, vol. 1: 85. 1753. Lectotype specimen: A specimen from the Hortus Cliffortianus in the British Museum of Natural History, London (BM; photograph, DAO!); specimen seen by Linnaeus.

The formula of the parentage of *T.* × *aestivum* L. is *Triticum turgidum* L. emend. × *T. aegilops* P. Beauv. ex R. & S. This interspecific hybrid species was selected as type of the genus *Triticum* L. by Hitchcock (15).

Description of Triticum × *aestivum L. emend.*—Spikes bearded or beardless, somewhat compressed, variable in width of faces and profiles; spikelets imbricate and crowded or more remotely spaced; each glume unicarinate with a sharply winged carinal adaxial vein, the wing indistinct towards the glume base, and a slightly raised lateral abaxial vein; lemmas awned or awnless.

Flaksberger (9) included the allohexaploid wheats in sect. SPELTOIDEA Flaksb., but since this section includes *T.* × *aestivum* L., the type of the genus *Triticum* L. emend., the section should be called sect. ×TRITICUM if sectional classification is used.

Groups of Cultivars

I am including six of the cultivated AABBDD allohexaploid wheats as groups of cultivars under *T.* × *aestivum* L. emend. If wild AABBDD allohexaploid wheats can be recognized, they should not be treated as subspecies, varieties, or forms, but rather as nothomorphs (hybrid forms) under Appendix I, article H.5 of the Botanical Code (18).

The groups of cultivars of *Triticum* × *aestivum* L. emend. are:

1. group aestivum
2. group spelta
3. group compactum
4. group sphaerococcum
5. group macha
6. group vavilovii

1. Group aestivum, l.c., the typical group, i.e., based on *T. aestivum* L., Sp. Pl., ed. 1, vol. 1: 85. 1753. Group aestivum, in the strict sense, appears to me to be morphologically the same as *T. vulgare* Host. var. *erythrospermum* Körn., described on page 276 of Percival (24), i.e. with bearded spikes, white glabrous glumes, white awns, and reddish grains. Many cultivated varieties belong here as cultivars and several subgroups may be required to classify them. The cultivars of spring wheats listed by Fraser and Whiteside (12) and the numerous cultivars of common wheat listed by Percival (24) and by Bayles and Clark (4) belong in group aestivum.

2. Group spelta. Based on *T. spelta* L., Sp. Pl., ed. 1, vol. 1: 86. 1753. Percival (24) listed numerous cultivars that belong in group spelta.

3. Group compactum. Based on *T. compactum* Host, Icon. et Descr. Gram. Austr. 4:4 and tab. 7. 1809. Many cultivars also belong in this group, including numerous cultivars listed by Percival (24).

4. Group sphaerococcum. Based on *T. dicoccoides* Körn. race *T. sphaerococcum* Perc., The Wheat Plant, pp. 157, 321–324 and Figs. 202–206. 1921. Percival (24) stated that sphaerococcum is cultivated in India and Iran and he listed some varieties (his page 323) that belong here as cultivars.

5. Group macha. Based on *T. macha* Dek. & Men., Bull. Appl. Bot. Gen. Pl. Br., ser. 5, No. 1: pp. 14–23; 38–44 and figs. 2–6. 1932. Nevski (22) suggested that this wheat was closely related to the tetraploid wheats such as *T. turgidum* group dicoccon. He considered that macha was an endemic cultivar of Western Georgia, U.S.S.R. When Dekaprelevich and Menabde originally described *macha*, they also described some botanical varieties at least some of which belong in group macha as cultivars.

6. Group vavilovii. Based on *T. vavilovi* Jakubz., Priroda (Nature), No. 11: 72–73. 1933. See also, *T. vavilovi* Jakubz. in Zhukovsky, La Turquie Agricole (Partie Asiatique – Anatolie). pp. 705–707 and 805. 1933. Syn.: *T. vulgare* Vill. *compositum* Tum. var. *vavilovi* Tum., Bull. Appl. Bot. Gen. Pl. Br., ser. 5, No. 2: 273, 281 and fig. 3. 1934. Several varieties belong here as cultivars.

A more detailed classification of all the allohexaploid wheat cultivars in these six groups should be undertaken by a wheat expert or by a group of experts.

II (c). Allopolyploid Species of Interspecific Hybrid Origin (2n = 28 or 42)

Kihara (17) demonstrated that some of these allopolyploid species have originated from natural hybridization between certain diploid species. Some genomes in these allopolyploids appear to be the same as in the diploids, but some genomes in some allopolyploid species seem to be modified. Of the 10 allopolyploid species, all are allotetraploid, $2n = 28$, except for the allohexaploid ($2n = 42$) strains of *T. triaristatum* and *T. crassum* and the allohexaploid *T. juvenale*. The formulae of parentages listed below are based on table 3 of Kihara (17).

1. *Triticum ovatum* (L.) Raspail, Ann. Sc. Nat. ser. 1(5): 435. 1825. Based on *Aegilops ovata* L., Sp. Pl., ed. 1, vol. 2: 1050. 1753. Parentage: *T. umbellulatum* × *T.* sp.? This species was designated as type of the genus *Aegilops* L. by Hitchcock (15*a*).

2. *Triticum triaristatum* (Willd.) Godr. & Gren. in Gren. & Godr., Fl. Fr. 3: 602. 1856. Based on *Aegilops triaristata* Willd., Sp. Pl. 4: 943. 1805. Parentage of the tetraploids: *T. umbellulatum* × *T. tripsacoides*.

3. *Triticum* **kotschyi** (Boiss.) Bowden, comb. nov., based on *Aegilops kotschyi* Boiss., Diagn. Pl. Or. Nov., ser. 1, fasc. 7: 129. 1846. Parentage: *T. umbellulatum* × *T.* sp.?

4. *Triticum triunciale* (L.) Raspail, Ann. Sc. Nat. ser. 1(5): 435. 1825. Based on *Aegilops triuncialis* L., Sp. Pl., ed. 1, vol. 2: 1051. 1753. Parentage: *T. umbellulatum* × *T. dichasians*.

Lectotype: Specimen of *Aegilops triuncialis* L., Savage (32) Catalogue No. 1218.8 (from Spain) in the Linnaean Herbarium, London (LINN; photograph, DAO!).

5. *Triticum cylindricum* Ces., Pass. & Gib., Comp. Fl. Ital., part 1, fasc. 4: 86. 1869. Syn.: *Aegilops cylindrica* Host, Gram. Austr. 2: 6 and tab. 7. 1802. Parentage: *T. dichasians* × *T. aegilops*.

6. *Triticum macrochaetum* (Shuttl. & Huet. ex Duval-Jouve) Richter, Plant. Europ. 1: 128. 1890. Based on *Aegilops machrochaeta* Shuttl. & Huet.

ex Duval-Jouve, Bull. Soc. Bot. France, 16: 384–385. 1869. Syn.: *A. biuncialis* Vis., Fl. Dalmatica 1: tab. 1, Fig. 2. 1842 and Fl. Dalmatica 3: 344. 1852. Non *T. biunciale* Vill., Hist. Pl. Dauph. 2: 167. 1787. Parentage: *T. umbellulatum* × *T.* sp.?

7. *Triticum crassum* (Boiss.) Aitch. & Hemsl., Trans. Linn. Soc., ser. 2, vol. 3: 127. 1888. Based on *Aegilops crassa* Boiss., Diagn. Pl. Or. Nov., ser. 1, fasc. 7: 129–130. 1846. Parentage of the tetraploids: *T. aegilops* × *T.* sp.?

8. *Triticum* **turcomanicum** (Rosh.) Bowden, comb. nov., based on *Aegilops turcomanica* Rosh, Bull. Appl. Bot. Gen. Pl. Br. 18: 413–415. 1928. Syn: *T. turcomanicum* Rosh., l.c., p. 413., pro syn., *nomen invalidum.*

My plants of *T. turcomanicum* were tetraploid, $2n = 28$.

9. *Triticum juvenale* Thellung in Fedde, Rep. Spec. Nov. 3: 281–282. 1907. Syn.: *Aegilops juvenalis* (Thellung in Fedde) Eig in Fedde, Rep. Spec. Nov. Beih. 55: 93–95. 1929. Kihara (17) indicated that *T. aegilops* was one of the three species in the parentage of *T. juvenale.*

10. *Triticum ventricosum* Ces., Pass. & Gib., Comp. Fl. Ital., part 1, fasc. 4: 86. 1869. Syn.: *Aegilops ventricosa* Tausch, Flora 20: 108. 1837. *A. squarrosa* sensu Cosson, Not. Quelq. Pl. 2: 68–69. 1849. *A. squarrosa* sensu Willd., Sp. Pl. 4: 944. 1805. Pro parte, typo Linnaei excluso. *A. fragilis* Parlat., Fl. Ital. 1: 515. 1848. *T. fragile* (Parlat.) Ces., Pass. & Gib., Comp. Fl. Ital., part 1, fasc. 4: 87. 1869. Non *T. fragile* Roth. Catal. Bot., fasc. 2: 7. 1800. Parentage: *T. aegilops* × *T.* sp. ?

When Tausch described *Aegilops ventricosa* Tausch, he was the first author to clearly show that more than one species was being classified by post-Linnaean botanists under *A. squarrosa* L. Tausch restricted the name *A. squarrosa* to the Central Asiatic species for which I have accepted the name *T. aegilops* P. Beauv. ex R. & S. Tausch described *A. ventricosa* but incorrectly gave the geographical range as "Habitat in Oriente". Cosson was the first author to describe correctly the geographical range of the western Mediterranean species, *T. ventricosum*, but Cosson applied the name *A. squarrosa* to this species and called the Central Asiatic species *A. tauschii* Cosson. Since Tausch made the first clear delimitation of Linnaeus' *A. squarrosa*, we must follow Tausch's treatment and reject that of Cosson.

There are several historical specimens that belong to *T. ventricosum* Ces., Pass. & Gib. but none of them can be used as type specimens. Three of the specimens labelled *A. squarrosa* in the Willdenow Herbarium, Berlin, belong to *T. ventricosum* (B; photographs, DAO!).

III. Other Artificial and Natural Interspecific Hybrids

Other interspecific hybrids belong here, for example some of the hybrids listed on page 226 of Eig (8) and some of the numerous hybrids listed by Percival (24), Sears (36), and Riley *et al.* (27). Ciferri and Giacomini (6) listed many formulae of interspecific hybrids (sensu *Triticum* L. emend. i.e. including *Aegilops* L.). They gave formulae of parentages and hybrid epithets, but unfortunately most and perhaps all of these hybrid names, certainly at least the new names, are invalid because they are *nomina nuda.*

Ciferri and Giacomini (6) listed these formulae and names on pages 49, 50, 51, 179, and 180 of their nomenclator. Under my new classification of the wheats and their allies, some of these formulae now apply to intraspecific hybrids rather than to interspecific hybrids under previous classifications.

It should be noted that names and epithets are invalid if they are *nomina nuda*, or if they lack Latin descriptions when they have been published after Jan. 1, 1935, or if the nomenclatural types are not indicated when they have been published after Jan. 1, 1958. To validate any of these names, they will have to be republished according to the Botanical Code (18).

The Taxonomy of the Barleys and Their Relatives

The biological and nomenclatural situation in *Hordeum* L. section Hordeum, which includes the cultivated barleys, is not as difficult to understand as the situation in the cultivated wheats.

The type species of the genus *Hordeum* L. is *H. vulgare* L. It was selected as type species by Britton and Brown (5) and was also designated as type species by Nevski (23). Nevski (23) wrote a remarkable monograph of the genus *Hordeum* and considered all of the species known at that time. He gave a thorough taxonomic treatment, keys, morphological descriptions, maps, drawings of the plants, and citations of specimens examined. He recognized six sections and included *H. vulgare* L. in sect. Crithe Doell. Åberg (1) discussed the cereal barleys and their wild relatives under sect. Cerealia Ands. Since sect. Crithe Doell and sect. Cerealia Ands. include *H. vulgare* L., which is the type of the genus *Hordeum*, the correct sectional name under the Code (18) is sect. Hordeum. In the present paper, I shall primarily discuss *Hordeum* L. sect. Hordeum since it includes all of the important cereal barleys.

Nevski (23) listed three species in sect. Hordeum (as sect. Crithe Doell), namely, *H. spontaneum* C. Koch, *H. distichon* L., and *H. vulgare* L. He treated the latter two species in detail and listed numerous additional species under each. Nevski (23) considered the distribution and phylogeny of the genus *Hordeum*. His map (his Fig. 7) shows that species of *Hordeum* occur in Eurasia and in North and South America. The center of origin was presumably in Eurasia, perhaps in the area from the eastern Mediterranean to Central Asia, and migration to the Americas was presumably by way of the Bering Straits. The distribution of the genus indicates that it had already been established in Eurasia and the Americas by at least the Tertiary Period since there are distinct species in the different geographical areas. On page 96 of Nevski (23), the relationships of the six sections are shown. The ancestral stock is shown as a hypothetical "*Protohordeum*" and from it have been derived two branches: five sections from one branch and sect. Hordeum (as sect. Crithe) in a separate branch. The important point to consider here is that all of the species of *Hordeum* usually are heterospiculate, that is, they have central fertile spikelets (bearing seeds) and reduced lateral spikelets (no seeds), except *H. vulgare* L. (including *H. hexastichon* L.) and *H.*

agriocrithon Åberg. In *H. vulgare* and *H. agriocrithon*, the spikelets are normally isospiculate with seeds in each of the three spikelets per node.

Several phylogenetic schemes have been proposed to show the relationships of the wild and cultivated barleys in sect. HORDEUM. Schiemann (33) considered that *H. agriocrithon* and *H. spontaneum* were the wild (spontaneous) barleys and that *H. vulgare* L. and *H. distichon* L. were the derived cultivated barleys. Schiemann (33) considered that *H. agriocrithon*, with a six-rowed, isospiculate, brittle rachis, was the ancestral species. Åberg (1) also thought that *H. agriocrithon* was the oldest species and showed several alternative hypotheses (his page 141) for the origin of the other barley species. Schiemann (34) discussed some mutants of *H. agriocrithon* and showed that this species has been found in three localities on the Plateau of Tibet: Lhasa (Tibet) and Tsela Dzong and Taofu (Sikang Province in Western China). The only other wild barley of sect. HORDEUM is *H. spontaneum*, which is a two-rowed, heterospiculate, brittle-rachis barley. In Fig. 69 of Nevski (23), the map of the natural range of *H. spontaneum* shows a definite distribution area from the eastern Mediterranean to Central Asia. It is a range pattern of a long-established wild species.

The evidence strongly suggests to me that an alternative hypothesis of the origin of the cultivated barleys should be seriously considered. All of the wild species of *Hordeum* are usually heterospiculate with the exception of the isospiculate *H. agriocrithon* which occurs on the Plateau of Tibet. The heterospiculate condition of *Hordeum* must have been established by at least the Tertiary Period. The question is: when did the isospiculate *H. agriocrithon* evolve? The cultivated *H. vulgare* and *H. distichon* have been derived by the selection practices of man. The ancestral stock of the genus *Hordeum* may have been derived, and probably was derived, from an isospiculate ancestor. But the genus *Hordeum* is basically heterospiculate and in my opinion the isospiculate condition of *H. agriocrithon* and *H. vulgare* is a derived and more recent condition and is undoubtedly due to gene mutation. Occasional specimens of *H. agriocrithon* have spikes that show a mixture of isospiculate and heterospiculate spikelets (Cyt. No. 2057 S2, DAO!).

I therefore consider that *H. spontaneum* represents the original wild species with primitive characteristics and that *H. agriocrithon* belongs to a mutant variety formed in the eastern part of the ancestral area. All of the other barleys of sect. HORDEUM have originated in cultivation but have presumably been derived from material originally brought from the wild. Many different combinations and recombinations of genes are represented in cultivated material.

From these observations and my unpublished data, the cereal barleys and their close relatives are known to belong to a diploid complex, $2n = 14$. The cytology and genetics of barley have been reviewed by Smith (38). The cultivated barleys have been developed from the original wild populations and the modern cultivars are the result of breeding and selection. In my opinion, only a single species is involved. I propose to treat the wild barleys of sect. HORDEUM as subspecies and varieties under the Botanical Code (18).

According to article 28 of the Botanical Code (18), the cultivars should be treated under the Horticultural Code (11) and this treatment greatly simplifies the nomenclature of barley since many species names have been applied to barley cultivars (see Åberg and Wiebe (2), Schiemann (33), and Nevski (23)).

The type of the genus *Hordeum* L. and one of the oldest specific names, *H. vulgare* L., is chosen to designate this species: *Hordeum vulgare* L., nom. emend., Sp. Pl., ed. 1, Vol. 1: 84. 1753. Lectotype specimen: Savage (32) Catalogue No. 103.1 in the Linnaean Herbarium, London (LINN; photograph, DAO!).

Description of H. vulgare L. emend.—Plants annual or winter annual; spike dense, coarse; three spikelets per node, isospiculate or heterospiculate; glumes lanceolate, fine-awned, glume backs usually sericeous, sometimes glabrate; lemmas of the fertile florets coarse-awned, or hooded or awnless in certain cultivars; seeds large. Nevski (23) listed other morphological characteristics under sect. CRITHE and Åberg and Wiebe (2) gave a description under sect. CEREALIA that applies to this species.

The infraspecific taxa of *Hordeum vulgare* L. emend. are:

1. subsp. *spontaneum*
   (*a*) var. *spontaneum*
   (*b*) var. *ischnatherum*
2. subsp. *vulgare* var. *vulgare* f. *agriocrithon*
3. Groups of cultivars:
   (*a*) group vulgare
      i. subgroup vulgare
      ii. subgroup trifurcatum
      iii. other subgroup
   (*b*) group distichon
      i. subgroup distichon
      ii. subgroup zeocriton
      iii. subgroup deficiens
      iv. other subgroup
   (*c*) group irregulare

1. *H. vulgare* L. emend. subsp. *spontaneum* (C. Koch) Thellung, Flor. Adv. Montpell., Mem. Soc. Nat. Sci. Nat. Math., Cherbourg, 38: 160–161. 1912. Based on *H. spontaneum* C. Koch, Linnaea, 21: 430–431. 1848.

(*a*) var. *spontaneum*, the typical variety of this subspecies, l.c. Type: "Auf den steppenartigen Matten des schirwan'schen Theiles des Kaukasus, 500–1000' hoch". This is the wild, heterospiculate barley with two-rowed, brittle rachis and with obtuse-apexed, awnless lemmas in the lateral spikelets (see Nevski (23)). Åberg (1) showed that there were many variations in var. *spontaneum* which appear to me to be forms.

(*b*) var. *ischnatherum* (Cosson) Thellung, Flor. Adv. Montpell., Mem. Soc. Nat. Sci. Nat. Math., Cherbourg, 38: 161. 1912. Based on *H. ithaburense* Boiss. var. *ischnatherum* Cosson, App. Flor. Juv. alt., Bull. Soc. Bot. France, 11: 163. 1864. This variety is restricted to the southwestern part of the

species range (see Nevski (23)). It is the wild, heterospiculate barley with two-rowed brittle rachis and with short, triangular, acuminate apices in the lemmas of the lateral spikelets (see Nevski (23)).

2. *H. vulgare* L. emend. subsp. *vulgare* var. *vulgare*, the typical subspecies and variety, l.c., i.e., based on *H. vulgare* L., sensu stricto.

(*a*) f. *agriocrithon* (Åberg) Bowden, comb. nov., based on *H. agriocrithon* Åberg, Ann. Agr. Coll. Sweden 6: 159–216. 1938. This is the wild, isospiculate, six-rowed, brittle-rachis barley now known from three stations on the plateau of Tibet (see Schiemann (34)).

3. Groups of Cultivars

I propose to treat the cultivars under article 28 of the Botanical Code (18) and under the appropriate articles, particularly article 13, of the Horticultural Code (11). In the discussion that I have already given on phylogeny, several different hypotheses are outlined but none of these have been proved beyond doubt, although Schiemann's paper (34) lends support to the idea that var. *vulgare* f. *agriocrithon* is the ancestor of the six-rowed, tough-rachis cultivated barleys. It is possible, as Åberg (1) has suggested, that the cultivated six-rowed and two-rowed barleys have had a polyphyletic origin. With our present knowledge of phylogeny, I think it best to classify the cultivars into several groups and subgroups under article 13 of the Horticultural Code (11).

The groups of cultivars of *Hordeum vulgare* L. emend. are:

(*a*) Group vulgare. Based on *H. vulgare* L., sensu stricto. This group includes the isospiculate, six-rowed, tough-rachis barley cultivars. At least three subgroups can be designated and more are probably needed to classify all of the cultivars known (see Åberg and Wiebe (2)).

i. Subgroup vulgare. Based on *H. vulgare* L., sensu stricto. This subgroup includes the six-rowed, tough-rachis barleys with long-awned lemmas. Many cultivated barleys belong here as cultivars (see Åberg and Wiebe (2)).

ii. Subgroup trifurcatum. Based on *H. coeleste trifurcatum* Schlecht; Linnaea, 11: 543–544. 1837. This subgroup contains the isospiculate, six-rowed, tough-rachis, hooded barleys.

iii. Another subgroup may be needed to include the awnless, six-rowed barleys.

(*b*) Group distichon. Based on *H. distichon* L., Sp. Pl., ed. 1, Vol. 1: 85. 1753. This group includes the heterospiculate, two-rowed, tough-rachis barley cultivars. In the earlier discussion of phylogeny, I have suggested that group distichon is closely related to *H. vulgare* L. subsp. *spontaneum* (C. Koch) Thellung. At least three subgroups can be designated and more may be required to classify all of the known cultivars.

i. Subgroup distichon. Based on *H. distichon* L., l.c. This subgroup includes the two-rowed, tough-rachis barleys with normal spikes. Many cultivated barleys belong here as cultivars (see Åberg and Wiebe (2)).

ii. Subgroup zeocriton. Based on *H. zeocriton* L., Sp. Pl., ed. 1, Vol. 1: 85. 1753. Lectotype specimen: Savage (32) Catalogue No. 103.2 in the

Linnaean Herbarium, London (LINN; photograph, DAO!). Subgroup zeocriton includes the two-rowed barleys with compressed, flabellate spikes.

iii. Subgroup deficiens. Based on *H. deficiens* Steud., Syn. Pl. Gram.: 351. 1855. This subgroup includes the two-rowed, tough-rachis barleys in which the lateral spikelets are much reduced and consist of small rudimentary glumes and other much reduced parts.

iv. The two-rowed hooded barleys belong in a separate subgroup.

(*c*) Group irregulare. Based on *H. irregulare* Åberg & Wiebe, Jour. Wash. Acad. Sc., 35: 161–164 and Fig. 1. 1945. This group includes the irregular barleys in which the spikes are composed of an irregular mixture of isospiculate and heterospiculate spikelets.

I have not attempted to give a complete classification of barley cultivars since this will require an extensive investigation and should be done by a barley expert or by a committee of experts. The classification of Åberg and Wiebe (2) should be a valuable guide in preparing a complete classification of barley cultivars.

Many other species names have been used for cultivated barleys but these barleys should be reclassified within the groups and subgroups of cultivars. Some of the species names that have been used for cultivars that have not been included in my classification above are: *H. intermedium* (Körn.) Carleton mentioned by Schiemann (33) and *H. nudum* (L.) Ard., *H. coeleste* (L.) P. Beauv., *H. hexastichon* L., and *H. revelatum* (Körn.) A. Schultz., mentioned by Nevski (23). All of these barleys are cultivars and should be included in the classification of groups and subgroups of cultivars and should not be treated in the botanical classification as subspecies and varieties.

It should be noted that Rode (28) gave two names under *H. distichon* L., namely "a) *erectum*" and "b) *nutans*". Nevski (23) credited Rode as having published var. *erectum* and var. *nutans* but page 26 of Rode (28) shows that he meant "a)" and "b)" to be subspecies. These taxa also belong to the classification of cultivars.

In the above treatment of the cultivated and wild barleys of *Hordeum* L. sect. HORDEUM, two important concepts have been followed. First, it is certain that all of these cultivars and the wild subsp. *spontaneum* (two vars.) and subsp. *vulgare* var. *vulgare* f. *agriocrithon* belong to a single species, *H. vulgare* L. There are certainly many gene mutations and many different combinations and recombinations of genes in the wild populations and especially in the cultivated material of this species. Second, the classification of the barleys of sect. HORDEUM is much simplified if the wild plants are classified under the Botanical Code (18) and the cultivars are placed in groups and subgroups under the Horticultural Code (11).

THE TAXONOMY OF THE RYES AND THE GENUS *Secale* L.

Because investigations on the taxonomy of *Secale* are incomplete, it is not my intention to present a complete treatment now. However, it seems useful to have a brief outline of the current taxonomic situation in this genus.

The biological and nomenclatural situation in the ryes and the genus *Secale* L. is somewhat similar to that in the barleys and *Hordeum* L. sect. HORDEUM. I have catalogued about 44 species names in *Secale*. Some of these names have been transferred to species in other genera but many of the names are synonyms. Schiemann (33) considered the systematics of *Secale*, the rye genus, and recognized five species. Roshevitz (29) wrote a valuable monograph of the genus *Secale* and recognized 14 species. He gave a detailed taxonomic treatment with a key to the species, a list of synonyms, descriptions and drawings of specimens of the species, and maps that show the geographical distribution patterns. Except for the artificial tetraploid rye (Tetra-Petkus Winter Rye), all of the material of *Secale* species that I have examined is diploid, $2n = 14$, and the chromosomes are always very large, which is a characteristic of the genus.

It seems highly probable that an intensive study of the 14 species of *Secale* recognized by Roshevitz (29) will show that a much smaller number of species should be recognized. The close relationship of some of the taxa is certainly suggested by the morphology of some of the specimens and by Roshevitz' maps. Some of the maps show species with well-defined geographical areas that are characteristic of wild species that have been long established in nature. Other species have only one station shown. *Secale africanum* Stapf. in Hook. was described from Cape Colony, South Africa, but Adamson and Salter (3) list only *S. cereale* L. and state that it is an escape from cultivation. Investigations are needed to establish how many species of *Secale* should be recognized.

The type species of the genus *Secale* L. is *S. cereale* L. It was selected as type by Hitchcock (15). Nevski (22) stated that "in the southern part of Central Asia and in Trans-Caucasia, *S. cereale* occurs in wild state as a weed in winter wheat fields". Of the species of *Secale* recognized by Roshevitz (29), *S. cereale* L. bears the oldest specific epithet. Some of the wild species of *Secale* probably belong in the same species as the cultivated ryes (*S. cereale*). Nevski (22) suggested that *S. vavilovii* Grossh. was closely related to *S. cereale* L. When the species and cultivars of rye are reclassified, *S. cereale* L. should be used as the name for the species that includes the cultivated ryes and their closest wild relatives. The cultivars should be treated under article 28 of the Botanical Code (18) and the appropriate articles of the Horticultural Code (11).

Acknowledgments

The author is indebted to many individuals and institutions for seeds, specimens, and literature, and for valuable discussions that have made the present paper possible.

Addendum

After the present paper was completed, reprints of papers by Heslot (42) and Heslot and Ferrary (43) were received.

Heslot (42) described *Triticum ispahanicum* Heslot which was collected at Ispahan (Isfahan) Iran. It is the new tetraploid ($2n = 28$) species referred to by Jakubziner (16). I have not yet grown plants of this taxon so cannot

treat its status in the present paper. Presumably the type is a cultivated specimen preserved at the Institut National Agronomique, Paris, France. Heslot (42) also described two varieties of this species, namely, var. *uniaristatum* Heslot and var. *biaristatum* Heslot, but he did not indicate which one is the typical variety (var. *ispahanicum*).

Heslot and Ferrary (43) described *Triticum timonovum* Heslot & Ferrary. *Triticum timonovum*, which is octoploid, $2n = 56$, was obtained under cultivation by the colchicine treatment of tetraploid *T. turgidum* L. emend. var. *timopheevii* (Zhuk.) Bowden. I have grown two lots of plants of the octoploid (*T. timonovum*) from seed supplied by Dr. P. M. Zhukovsky of Leningrad, who obtained his original seeds from Dr. Heslot. The name *T. timonovum* Heslot & Ferrary was published with a Latin description but a type specimen was not cited as required by article 35 of the Botanical Code (18). Therefore this name is not yet validly published. Four specimens of this octoploid have so far been preserved in our herbarium (DAO).

Since *Triticum timonovum* has been derived by colchicine treatment of tetraploid *timopheevii* under cultivation, it is my opinion that it is not a new species but rather a cultivar under *T. turgidum* L. emend. var. *timopheevii* (Zhuk.) Bowden. This is clearly provided for in article 28 of the Botanical Code (18). According to article 15 of the Horticultural Code (11), for new cultivars (after January, 1959), "the name of a cultivar (variety) must be a fancy name, that is, one markedly different from a scientific name of Latin form". According to the Code (11), certain steps will have to be taken later to publish a cultivar name for this octoploid. The genomic formula appears to be AAAAGGGG and it is an auto-allooctoploid.

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SNOW MOLD INFECTION OF ALFALFA, GRASSES, AND WINTER WHEAT BY SEVERAL FUNGI UNDER ARTIFICIAL CONDITIONS[1]

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Abstract

Snow mold infection by an unidentified low-temperature basidiomycete and *Typhula* spp. developed best under artificial conditions when inoculated plants in pots were incubated for 2 months at 2° C with 80 to 90% R.H. in the special cabinets described or with other covering. A saturated atmosphere was detrimental to mycelial growth and infection. Freezing temperatures and complete host dormancy were not required but greenhouse-grown plants benefited from prior conditioning for 2 weeks at 2° to 5° C with light for 8 hours daily. The reactions of alfalfa varieties, creeping red fescue, Kentucky bluegrass, red top, timothy, and winter wheat to the low-temperature basidiomycete were similar to those previously observed in the field. *T. idahoensis* Remsb. caused moderate to severe damage and *T. itoana* Imai caused slight to moderate damage to the grasses and winter wheat. *Typhula* sp. from alfalfa was highly virulent to that host. The low-temperature basidiomycete was the only snow mold organism tested that synthesized HCN in culture or in the plant tissues.

Sclerotinia borealis Bub. & Vleug. caused slight damage to winter wheat and grasses after 3 months at 2° C but did not develop well under any of the conditions employed.

Introduction

Previous studies (1) have shown that there is great variation from year to year in the amount of snow mold damage caused by an unidentified low-temperature basidiomycete in Western Canada. This may be largely due to the influence of temperature, moisture, snow cover, and other factors on disease development. Since accurate information on these factors and their interaction could not be obtained in the field, the disease was studied under artificial conditions in the controlled temperature rooms at Lethbridge.

Comparative studies were made under the same conditions on infection by other important snow mold pathogens. *Typhula* spp. and *Sclerotinia borealis* were studied because they are widely distributed and cause damage that is markedly influenced by environmental conditions (3, 4, 5, 7, 10). Attempts were made to determine the optimum requirements for infection by each of these fungi. Studies were also made on their host relationships under these conditions and on the possible production of HCN by various snow mold fungi, as previously reported for the low-temperature basidiomycete (6).

Materials and Methods

Fungi and Inoculum

The snow mold pathogens and isolates studied were as follows:

Low-temperature basidiomycete: Isolates from alfalfa, Alberta and Manitoba.

[1]Manuscript received April 20, 1959.

[2]Canada Agriculture Research Station, Saskatoon, Saskatchewan.
[3]Plant Pathology Section, Canada Agriculture Research Station, Lethbridge, Alberta.

Typhula idahoensis Remsb.: Isolate from grass of Remsberg (8) and isolate from *Agrostis* sp., Ottawa, Ontario.

T. itoana Imai: Isolate from grass of Remsberg (8) and isolate from winter wheat, Armstrong, B. C.

Typhula sp. (possibly *T. trifolii* Rostr. (10)): Isolate from alfalfa, Ottawa, Ontario.

Sclerotinia borealis Bub. & Vleug.: Isolates from *Festuca* sp., Prince George, B. C., and from winter wheat, Vanderhoof, B. C.

Inoculum of the low-temperature basidiomycete was grown at 15° C in flasks of a sterilized 9:1 volume mixture of sandy soil and corn meal. *Typhula* spp. and *S. borealis*, which did not thrive in the soil medium, were grown at 10° C in a sterilized 9:1 volume mixture of oat hulls and corn meal moistened with a 2% dextrose solution.

Inoculations were made by placing 40 cc of pulverized inoculum on the surface of the soil near the crowns of the plants in each 6-in. pot. In all experiments four replicate pots of each host were inoculated with each pathogen and four were left uninoculated as controls.

Host Plants

Host plants and varieties used in most experiments were as follows:

Alfalfa (variety Grimm)
Creeping red fescue (*Festuca rubra* L.)
Kentucky bluegrass (*Poa pratensis* L.)
Red top (*Agrostis alba* L.)
Timothy (*Phleum pratense* L.)
Winter wheat (variety Kharkov)

All plants were grown in the greenhouse except in preliminary tests with hardened plants from the field. The seeds were planted in 6-in. pots of unsterilized sandy soil. Shortly after emergence the seedlings were thinned to 15 per pot for alfalfa and winter wheat and 20 per pot for the grasses. Experiments were usually started when the plants were about three months old.

Snow Mold Cabinets

Special cabinets were designed and built on the basis of results obtained in preliminary snow mold experiments in which various types of container and cover were used. They consisted of tightly constructed wooden boxes which could be stacked in the controlled temperature rooms to conserve space (Fig. 1). Each box 5 ft long, 2 ft wide, and 1 ft high held 20 well-spaced 6-in. pots. These were placed in a galvanized iron tray fitted in the bottom of the box. Water was added to this tray as required and the relative humidity was controlled by means of shuttered vents in both ends of the box. The boxes had side-opening hinged doors to permit examination and removal of the pots.

A glass-covered lid was placed on the top box in the series so that the plants could receive light, controlled by a time clock, as required during the conditioning period. A light intensity varying from 200 to 400 ft-c was provided

by raising or lowering a bank of fluorescent and incandescent lights held on a stand over the top cabinet. Room temperatures and relative humidities within the cabinets were recorded automatically.

After conditioning in the top cabinet, the plants were moved down into one of the unlighted lower cabinets for varying periods of incubation for disease development. Notes on mycelial growth (Figs. 1, 2) were taken at weekly intervals. The pots were placed in the greenhouse for 2 weeks before final notes were taken on snow mold infection and recovery of each plant.

Experimental Results

Factors Influencing Disease Development

Under natural conditions snow mold development is influenced by various factors affecting the host, pathogen, and infection. In the first place, the disease normally develops in plants that have been gradually hardened under changing environmental conditions in the late fall. Mycelial growth and infection by snow mold fungi also may be influenced by temperature, moisture, cover, and other factors (1, 3, 4, 5, 6). In this study, attempts were made to determine the influence of various factors on each of the progressive stages leading to disease development by different pathogens.

Temperature and Light Requirements

First consideration was given to temperature and light requirements for conditioning of the greenhouse-grown plants used in most experiments. This was necessary so that they could withstand the long incubation period at low temperatures required for disease development. The effect of subfreezing temperatures on the control and inoculated plants was also studied.

Grimm alfalfa inoculated with the low-temperature basidiomycete was used in most of the experiments, but some were repeated with winter wheat and grasses. The plants were exposed successively to 10°, 5°, 0°, −5°, and −10° C (±0.5° C). At temperatures above 0° C, exposures of the plants for 8 hours daily to artificial light (200–300 ft-c) and to natural light in the greenhouse were compared with no light. After the conditioning period, the control and inoculated plants were covered and held for 8 weeks at 2° to 5° C.

Alfalfa plants were exposed to 1-week periods at each temperature unless otherwise stated. The results, expressed as average percentages of dead or severely damaged plants, were as follows:

Treatment		Control	Inoculated
1	10°, 5° (artificial light); 0°, −5°, −10° C (4 days)	100%	100%
2	10°, 5° (artificial light); 0°, −5° C	18%	100%
3	10°, 5° (no light); 0°, −5° C	60%	100%
4	10°, 5° (artificial light); 0° C	None	90%
5	10°, 5° (greenhouse light); 0° C	None	90%
6	10°, 5° (no light); 0° C	20%	100%
7	10°, 2° C (artificial light, 2 weeks)	None	100%
8	2° to 5° C range (artificial light, 2 weeks)	None	100%
9	2° to 5° C range (no light)	15%	100%
10	5° C (artificial light, 2 weeks)	None	50%

The physical damage evident in the control plants exposed to subfreezing temperatures (treatments 1, 2, and 3) indicates that the plants were not sufficiently hardened to withstand such conditions. Furthermore, as in the studies of Lebeau and Dickson (6), freezing and complete dormancy of the host were not prerequisite to infection by the low-temperature basidiomycete.

The plants were conditioned most satisfactorily at 2° C or 2° to 5° C with artificial light (treatments 7 and 8). Light aided in the conditioning at all temperatures studied and natural light in the greenhouse did not give better results than the more convenient treatment with artificial light. Grasses and winter wheat reacted similarly to alfalfa in the conditioning tests except that they suffered less damage at subfreezing temperatures.

All the fungi studied have been reported to grow at 0° C and lower temperatures (1, 3, 4, 10). This enables them to develop and cause damage beneath a snow cover. To determine the most suitable temperature for mycelial growth under artificial conditions, inoculum was placed on the surface of unsterilized soil in pots. The pots were covered and placed at temperatures of 0°, 2°, 5°, and 10° C. Growth of all fungi studied started first at 10° C but slowed up after the first week, possibly due to antagonism from other soil microorganisms at this temperature. After 2 weeks, mycelium of the low-temperature basidiomycete and *Typhula* spp. was most abundant at 2° and 5° C (Fig. 2) and was also well started at 0° C. Growth of *S. borealis* was relatively slow at all temperatures. In subsequent experiments, inoculations with all the fungi were made at the start of the conditioning period at 2° C. The light supplied for 8 hours daily had no apparent effect on growth.

The temperature of 2° C used for conditioning of the plants and for mycelial growth of the fungi also proved generally suitable for the additional period required for disease development. Alfalfa inoculated with the low-temperature basidiomycete, conditioned at 2° to 5° C with light for 2 weeks and incubated at different temperatures for 8 weeks, had the following percentages of dead or severely damaged plants:

93% at 0° C,	100% at 2° to 5° C,
100% at 2° C,	60% at 5° C.

Incubation in a room varying between 2° and 5° C often gave as good results as at 2° C but infection was usually reduced at 5° C. In addition, the

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Fig. 1. Snow mold cabinets in controlled temperature room, showing top section under bank of lights and three lower sections with side-opening doors.

Fig. 2. Mycelial growth of low-temperature basidiomycete (left) and *T. idahoensis* (right) in pots of winter wheat after 2 weeks at 2° C.

Fig. 3. Snow mold development by the low-temperature basidiomycete in pot of Grimm alfalfa after incubation for 2 months at 2° C.

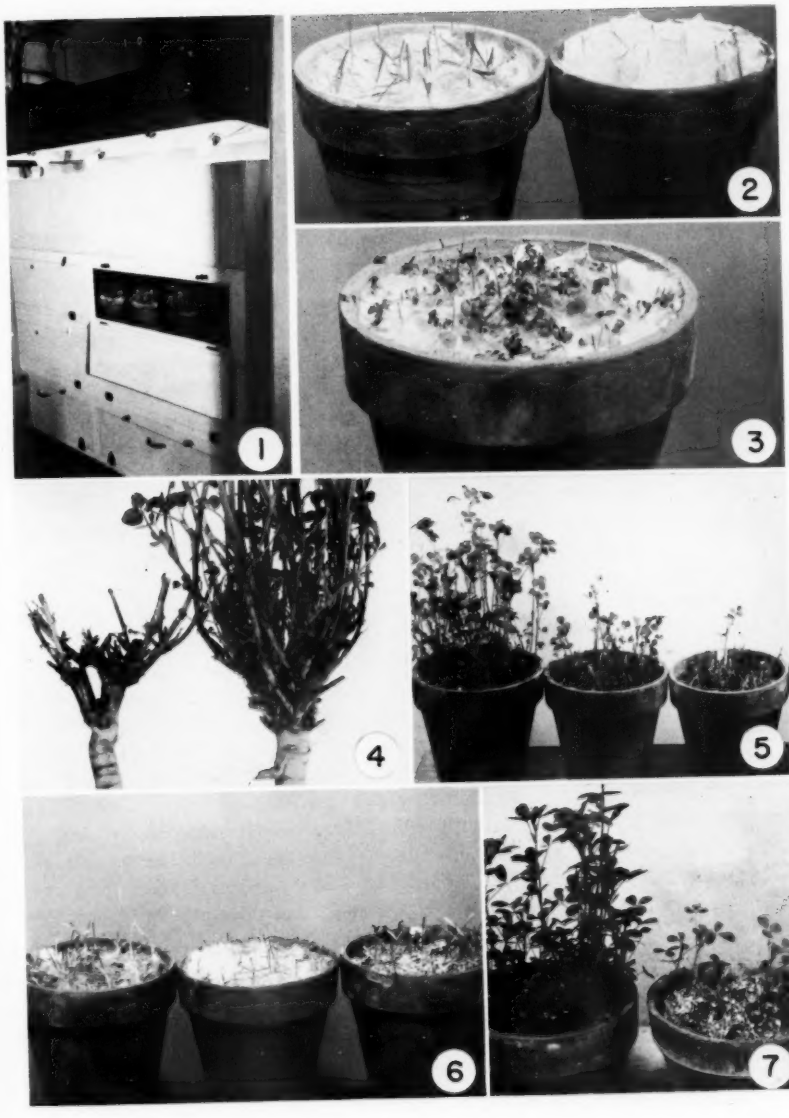
Fig. 4. Crowns of Grimm alfalfa plants: severely damaged by low-temperature basidiomycete under artificial conditions (left), uninoculated control (right).

Fig. 5. Varying degrees of susceptibility in alfalfa strains inoculated with the low-temperature basidiomycete under artificial conditions.

Fig. 6. Snow mold damage in pots of winter wheat inoculated with different pathogens, left to right: *T. itoana*, *T. idahoensis*, and *S. borealis*.

Fig. 7. Snow mold damage caused by *Typhula* sp. in inoculated Grimm alfalfa (right) and control (left).

PLATE I



plants were less dormant at 5° C, and under the dark conditions, produced a weak, spindly growth which was sometimes injured in both the control and inoculated series. Otherwise, no damage occurred in the controls.

Typhula spp. also caused more infection at 2° C than at higher and lower temperatures. However, disease development was usually less rapid than with the low-temperature basidiomycete. For example, in one experiment with alfalfa at 2° C the percentages of dead or severely damaged plants were as follows:

T. trifolii: 5 weeks, 20%; 8 weeks, 100%;
low-temperature basidiomycete: 5 weeks, 75%; 8 weeks, 100%.

At 0° C both fungi required more than 12 weeks to cause 100% damage.

Moisture and Cover Requirements

Moisture requirements for mycelial growth and disease development by the different pathogens were studied. In previous studies with the low-temperature basidiomycete (1) infection was reduced at a high soil moisture. To determine the effect of atmospheric humidity, the fungus was grown at 15° C on agar in open tubes enclosed in containers in which relative humidities of approximately 25, 50, 65, 75, 90, and 100% were maintained by means of a series of salt solutions (9). Growth of the three isolates studied was equally good at all except 100% R.H., where it was greatly retarded. In all experiments with the low-temperature basidiomycete and *Typhula* spp., mycelial growth and infection were reduced in a saturated atmosphere.

Under natural conditions a snow cover apparently aids in maintaining a favorable humidity for snow mold development (1, 3, 4, 5). However, since snow is not essential (1), other types of cover were studied, including glass and plastic sheets, pot saucers, and cabinets. Good results were obtained by covering the pots with inverted pot saucers and stacking them in piles covered with wax paper. This prevented drying of the surface soil and encouraged mycelial growth. Plastic bags and other tight covers produced saturated conditions which were particularly deleterious to the low-temperature basidiomycete and *Typhula* spp. *S. borealis* was less affected by these conditions.

In experiments in the snow mold cabinets at 2° to 5° C and 80 to 90% R.H. the low-temperature basidiomycete and *Typhula* spp. grew well and caused severe infection in open pots of susceptible hosts (Figs. 2, 3). Mycelial growth and infection were retarded when the pots were covered with pot saucers and were greatly reduced by covering with plastic.

Influence of Host Age and Vigor

Alfalfa and grass seedlings under 3 weeks of age proved less susceptible to snow mold damage than older plants. They were also less easily conditioned and the controls usually suffered physical damage during the long incubation period.

The degree of conditioning required by plants prior to incubation for snow mold development was influenced by stage of growth and other factors. Partially hardened alfalfa plants taken from the field in the late fall could be

immediately inoculated and incubated in the dark at 2° C. Such plants could also be stored at a low temperature for several weeks. Conversely, plants taken from the field in the early spring shortly after the soil thawed were particularly susceptible to cold injury and were less satisfactory than greenhouse-grown plants for snow mold tests.

Weak or injured plants were particularly susceptible to snow mold infection. In one experiment, Grimm alfalfa plants moderately infected with bacterial wilt, caused by *Corynebacterium insidiosum* (McCull.) Jensen, were more severely damaged by the low-temperature basidiomycete than plants not infected with wilt.

Influence of Organic Additives

Corn meal was used in the inoculum in the present study and in previous experiments (1, 2) to stimulate growth of the pathogens. Lebeau and Dickson (6) showed that HCN production was increased and plant survival was reduced when alfalfa crown meal, corn meal, and soybean meal were mixed with inoculum of the low-temperature basidiomycete. In further experiments with this fungus and *Typhula* spp. the addition of 25 cc per pot of sterilized alfalfa meal or corn meal stimulated mycelial growth and resulted in an average of 20% more infected plants than without additives. Soybean meal did not have a consistently stimulatory effect and it sometimes caused physical injury to the plants. The additives had no effect when unsterilized.

Host Relationships of Fungi

The relative virulence of the snow mold pathogens on different host plants was studied in the controlled temperature rooms at 2° to 5° C. In three experiments at least 150 plants of each host were inoculated with each pathogen. Table I shows the average amounts of infection and damage caused by the low-temperature basidiomycete and *Typhula* sp. during 2 months' incubation and by *S. borealis* during 3 months.

As in field experiments (2), the low-temperature basidiomycete was most virulent on Grimm alfalfa (Fig. 3), creeping red fescue, red top, and winter

TABLE I

Reaction of alfalfa, grasses, and winter wheat to snow mold pathogens under artificial conditions at 2° to 5° C (mean of three experiments)

Host	Average % of plants infected and damage caused by:				
	Low-temperature basidiomycete	*Typhula idahoensis*	*T. itoana*	*Typhula* sp.	*Sclerotinia borealis*
Alfalfa (Grimm)	95, Sev.	0	0	90, Sev.	0
Creeping red fescue	90, Sev.	100, Sev.	70, Mod.	10, Sl.	40, Sl.
Kentucky bluegrass	50, Mod.	95, Mod.	60, Mod.	15, Sl.	35, Sl.
Red top	100, Sev.	100, Sev.	70, Mod.	10, Sl.	45, Sl.
Timothy	70, Mod.	85, Mod.	40, Sl.–Mod.	10, Sl.	10, Sl.
Winter wheat (Kharkov)	80, Sev.	95, Sev.	35, Sl.–Mod.	5, Tr.	30, Sl.

wheat. The symptoms on alfalfa (Fig. 4) and other hosts were similar to those produced in the field. No infection was obtained in limited tests on brome grass (*Bromus inermis* Leyss), which proved highly resistant under field conditions (2).

Alfalfa varieties inoculated with the low-temperature basidiomycete in the cold rooms reacted as in the field (2). Rhizoma and Viking were less susceptible than Grimm, and strains of *Medicago falcata* L. were highly resistant. Alfalfa strains tested were also damaged to varying degrees (Fig. 5).

T. idahoensis was highly virulent on creeping red fescue, red top, and winter wheat (Fig. 6) and caused moderate damage to Kentucky bluegrass and timothy. Numerous dark-brown sclerotia, of the type described by Remsberg (8), were embedded in the dead stems of these grass hosts. Alfalfa was not attacked. *T. itoana* was more variable in virulence and caused less damage than *T. idahoensis* to all hosts studied (Fig. 6). Light-brown, relatively large sclerotia, as described by Remsberg (8), were sparingly produced in the dead stems of some of these hosts.

Typhula sp. from alfalfa, unlike the other species studied, caused severe damage to alfalfa in all experiments (Fig. 7). Small black sclerotia developed in the base of some of the dead stems. This isolate, which resembles published descriptions of *T. trifolii* (10), is apparently specific to legumes since it caused only trace to slight infection in grasses and winter wheat (Table I).

S. borealis caused slight damage to the grass hosts and winter wheat (Fig. 6), but did not attack alfalfa. No sclerotia were produced in the diseased tissues. Conditions favorable for the maximum development of this pathogen were not found during these experiments.

HCN in Relation to Disease Development

The unusual combination of conditions necessary for the development of snow mold by various pathogens in the field and in controlled temperature rooms has been difficult to explain. The disease is incited at temperatures near the freezing point, which are far below those for optimum growth of the snow mold pathogens (3, 4, 6, 10). In studies on the low-temperature basidiomycete, Lebeau and Dickson (6) showed that low temperatures favored maximum absorption by the host tissues of HCN produced by the fungus. Since the association between the production of HCN by this pathogen and the etiology of the disease has been established, it was decided to carry out a similar investigation on some of the other snow mold pathogens to determine if the same mechanism of pathogenesis was evident.

In culture studies, *T. idahoensis*, *T. itoana*, *Typhula* sp. from alfalfa, *S. borealis*, and the low-temperature basidiomycete were grown for 60 days at 15° C in 200-ml Erlenmeyer flasks on the oat hull – corn meal medium. The contents of each flask were steam-distilled and HCN determinations were made as described by Lebeau and Dickson (6). Positive tests for HCN were obtained for the low-temperature basidiomycete but not for the other fungi.

In studies on the host tissues, alfalfa buds from plants infected with low-temperature basidiomycete in the snow mold cabinets yielded over 600 p.p.m.

HCN. However, alfalfa and winter wheat infected in the same cabinets by *Typhula* sp. from alfalfa and *T. idahoensis*, respectively, gave negative tests when the diseased tissues were analyzed for HCN.

These results suggest that the low-temperature basidiomycete may be the only one of these snow mold pathogens that synthesizes HCN in culture or in plant tissues. The production of this lethal substance probably accounts for the wide host range of the low-temperature basidiomycete (2) because most of the tissues of higher plants are sensitive to HCN. *Typhula* spp. and *S. borealis* are more specific in their host relationship so it is not surprising that cyanide synthesis was not detected from any of these pathogens.

Discussion

The marked influence of environmental factors on snow mold development was very evident during the present study. Under artificial conditions, more control of temperature, moisture, and other factors was possible than in the field. Also, the same general conditions could be maintained for conditioning of the host plants, mycelial growth of the fungi, and disease development. These progressive stages interacted but the successful completion of one did not necessarily lead to another if conditions were not favorable. For example, abundant mycelial growth did not result in infection except at low temperatures. Different snow mold pathogens also differ in their response to environmental factors (3, 4, 5). In this study, the low-temperature basidiomycete and *Typhula* spp. caused damage under a fairly wide range of conditions but *S. borealis* developed poorly under all of the conditions employed. This may explain why most snow mold fungi have a much wider distribution than *S. borealis*, which has been reported only from extreme northern areas (3, 4, 7).

Low temperature appears to be the most important single factor favoring snow mold development. The disease develops under natural conditions on hosts that have been hardened gradually in the late fall at reduced temperatures. Similarly, artificial conditioning of the host at a low temperature was necessary for maximum disease development. Although most snow mold fungi grow best at temperatures ranging from 10° to 17° C, they also develop well at 0° C and cause damage only at low temperatures. With the low-temperature basidiomycete, this is apparently the result of increased absorption of HCN by the host at low temperatures (6). Since HCN production has not been demonstrated for other snow mold fungi some other mechanism may explain their low-temperature relationship.

The moderately moist conditions required for snow mold development are probably maintained by snow cover in nature. Under artificial conditions a vented cabinet or permeable covering served this purpose. A saturated atmosphere, however, was unfavorable for the development of the low-temperature basidiomycete and *Typhula* spp. This may explain the reduced snow mold damage in some seasons, especially under wet soil conditions (1).

In addition to providing basic information, artificial snow mold tests can have more practical purposes. Host and varietal tests for resistance to snow

mold pathogens can be conducted if space and facilities are available. These tests can be completed in 3 to 4 months at any season as compared with 1 year required under field conditions. Wernham and Chilton (11) also described a method for testing the reaction of grasses and cereals to *Typhula* spp. In the present study the reactions of alfalfa varieties and progenies to the low-temperature basidiomycete were comparable to those obtained in the field (2). Experiments under artificial conditions should also expedite the preliminary testing of materials for chemical control of snow mold.

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NUTRITIONAL STUDIES ON THE GENUS HIRSUTELLA

I. GROWTH RESPONSE IN AN ENRICHED LIQUID MEDIUM[1]

D. M. MacLeod[2]

Abstract

Quantitative comparative studies on the growth of *Hirsutella gigantea* in shaken yeast extract – dextrose medium have shown that the rate of growth and total yield is affected by the quantity of inoculum. The highest yield is obtained with an inoculum level ranging from 0.2 to 0.4 mg/50 ml; moreover results from repeated experiments tend to be relatively uniform. With smaller quantities of inoculum, i.e. 0.1 mg or less, the uniformity of growth among replicates is affected. An increase in the amount of inoculum above the optimum, while accompanied by a higher rate of growth, results in a gradual decrease in the total yield.

With approximately 0.2 mg of inoculum the maximum yield, 673.4 mg of fungal mycelium, was obtained after 12 days' incubation in a medium containing 1.5% yeast extract and 2% dextrose.

The fungus does not grow in a basal dextrose–salts medium with potassium nitrate or ammonium nitrate as the nitrogen source. The effectiveness of yeast extract indicates a requirement for organic nitrogen. Yeast extract may also act as a source of growth factors.

Introduction

Investigations on the nutrition and physiology of entomogenous fungi are still few in number, although this widespread and important group of fungal parasites has been the subject of investigation ever since man began to associate microorganisms with disease (17, 55). Much of the work in the past has been directed principally toward the identification of the various species, and in the case of the parasitic forms, to their dissemination in the field.

Undoubtedly the lack of progress in investigating the physiology of these organisms can be attributed in part to difficulties in obtaining and maintaining them in the laboratory in pure culture for long periods of time. Their failure to grow may be due to the absence of essential nutrients that are available only from their natural hosts.

Because of the scarcity of precise data on the nutritional requirements of these entomogenous forms, an investigation has been started on the nutrition, metabolism, and sporulation of a *Hirsutella* species that has been established in the laboratory outside of its host on a complex organic medium (32). The special aim in this investigation is to determine the nutritional requirements of this species, and by so doing possibly to throw some light on the culture requirements of the large number of fungi that grow almost exclusively on the cuticle or within the body cavity of living insects or mites. Moreover, a knowledge of their essential growth characteristics may well serve as a basis for an understanding of the nature of the host–fungus relationship. This is important because even today it is not generally known whether a fungus associated with a given host plays a parasitic or a saprophytic role.

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The results presented in this paper form an introduction to a study of the nutritional requirements of a *Hirsutella* species isolated from the spruce budworm; a short summary of the relevant literature on entomogenous fungi in general has also been included.

Literature Review

Bassi, who is regarded by many as the founder of the doctrine of pathogenic microbes or the germ theory of disease, is credited (55) with being the first to suggest employing microbial life to destroy insects harmful to man's interest. Following his infectivity experiments in 1834, others (2, 14, 46, 57) reported as successful attempts to transmit the infectious agent (*Beauveria bassiana* (Bals.) Vuill.) of muscardine to insects other than the silkworm. Thereafter, the idea gradually became established that certain fungi could be considered as natural enemies of insects, and in fact were responsible for various disease outbreaks among insects in nature (7, 11, 21, 22, 27, 36).

According to Steinhaus (55), Bail in 1865 exhibited a mold grown on a mash, and believed by him to be capable of killing such insects as flies, mosquitoes, and caterpillars. Later, in 1879, beer mash or diluted yeast was recommended by Hagen (25) in the United States as a means of controlling noxious insects. While it was eventually concluded that these organisms were non-pathogenic, such reports or observations are of some significance in that they may have influenced the use of artificial media as substrate for the growth of pathogenic forms. Subsequently, beer mash was used by Metchnikoff (37) for the mass production of spores for field distribution of the green muscardine fungus, *Metarrhizium anisopliae* (Metch.) Sorokin, parasitic on *Anisoplia austriaca* Hbst.

As a result of the stimulus of Metchnikoff's observations and recommendations, various methods of producing spores in quantity for field distribution have been devised. It has been found that although a number of entomogenous forms offer no particular difficulty from the standpoint of establishment on artificial media, they do not always sporulate (5, 6, 28, 32). Most of the formulations are kinds of complex natural media: soybean mash, corn meal agar, potato-dextrose agar, steamed rice, sterilized string beans, molasses agar, gelatine, beef-malt agar, proteose peptone medium, Sabouraud maltose agar, Molisch culture medium, casein hydrolyzate medium, Czapek agar (Dox and Thom's modification), Wort agar, bread (acid, alkaline, or neutral in reaction), potato and oatmeal agar with an admixture of trypsin, peptone, or ovomaltine, and of ground ants (4, 16, 23, 32, 42, 43, 44).

Because of the potential economic importance of the genera *Massospora* and *Empusa* (=*Entomophthora*) (belonging to the phycomycetous order Entomophthorales) as insect pathogens, many attempts were made, with little success among the earlier endeavors, to establish them on artificial media. Consequently obligate parasitism has been assumed to be predominant in this group. According to Speare (54) and Sawyer (48), *Massospora* has never been grown upon any artificial medium. In more recent years, however, some

degree of success (33, 49, 58) has attended attempts to culture *Empusa* species and it now seems probable that many of them will be found to develop saprophytically on natural media. Probably the most extensive studies concerning the growth of this group are those of Sawyer (48). He successfully cultivated *Empusa* (= *Entomophthora*) *sphaerosperma* Fresenius from the yellow-headed fireworm (*Acleris minuta* (Rob.)) on over 40 different natural media, including swordfish, pork, potato, and other natural media rich in proteins. It was concluded that neither carbohydrates nor fats were essential for growth.

Schweizer (49) has shown that following cold sterilization[3] the fly parasite *Empusa muscae* Cohn can be grown satisfactorily on meat extract gelatine with an admixture of blood or serum and glucosamine (a constituent of chitin). He also found that fly fat exerts a stimulating effect on the growth of young *Empusa* mycelium and on conidial germination. For germination, however, resting spores (azygo- and zygo-spores) are believed to require a special biocatalytic stimulus (49) which may be simulated in the laboratory by pure cultures of chitin-splitting bacteria.

The first instance in which a parasitic species of the Entomophthorales has been cultivated on a medium of defined chemical composition was reported by Wolf (58). He found that *Empusa* (=*Entomophthora*) *apiculata* Thaxter and *Empusa* (=*Entomophthora*) *coronata* (Cost.) Kevorkian can be grown upon a dextrose–asparagine–salts synthetic medium. Both species are autotrophic with respect to vitamins and other accessory growth factors. In a more recent paper Smith (51) also revealed that *E. coronata* grows well in a purely synthetic medium containing mineral salts, arginine hydrochloride, dextrose, and distilled water.

An indication of vitamin deficiency among some entomogenous fungi was reported by Benham (5), who found that although strains of *Beauveria tenella* (Delacro.) Siem. (= *B. densa* Link) failed to grow, or grew slightly on casein hydrolyzate medium and on Czapek agar, a decided increase in growth resulted when thiamine hydrochloride was added to the medium.

De Reaumur (15) in 1726, according to Steinhaus (55), recorded the first fungus associated with insects. It is perhaps noteworthy that this organism was a parasitic form belonging to the genus *Cordyceps* found on a noctuid larva thought to be an *Agrotis* species. Today 137 species are recognized in this genus (26). While subject to investigation for many years, very few attempts to establish any species of *Cordyceps* in culture have been successful. Sopp (52) appears to be the first botanist to succeed in obtaining a perfect stroma of *Cordyceps* on artificial media. He used malt extract agar, boiled meat, coagulated colostrum, or agar and gelatine mixed with peptone, urine, and a pea or soybean extract. Coagulated colostrum gave the best results. According to him several sclerotia were produced that developed stromata after a resting period. Later Kobayasi (26) using rice medium also showed that several species of *Cordyceps* could be grown saprophytically on various natural media.

[3]Shell No. III. (boiling point 38° C) was reported to be the best sterilization agent. It is composed essentially of paraldehyde, methylal, ethyl chloride, and ethyl mercaptan. An interval of 5 hours was recommended for sterilization.

Laboulbeniales, like *Cordyceps*, consists of a fungal group assigned to the Ascomycetes. All the 1500 described species are obligate parasites growing on the cuticle of living insects or mites. The Laboulbeniales are non-pathogenic parasites usually said (45) to cause no deleterious effect on the host. However, a substantially broader understanding of this group than now exists will be gained, according to Shanor (50), by solving the problem of how to grow at least some members of the order outside their insect hosts.

Material and Methods

I. Origin and Identity of Isolate

The genus *Hirsutella*, containing forms that grow within and on the bodies of larval and adult insects, was first described by Patouillard (40), in 1892, in an article entitled "Une Clavariée Entomogène". It was based on a species, *H. entomophila*, described from an undetermined beetle. Patouillard regarded the fungus as a Basidiomycete and included it in the Clavariaceae. In 1920, after a study of *H. entomophila*, Speare (53) concluded that the genus belongs in fact to the order Moniliales, Fungi Imperfecti.

The genus *Hirsutella* comprises 33 named species. A study of this group, based mostly on North American collections, has been recently published by Mains (34).

Under natural conditions, *Hirsutella* species have been reported from spiders and other Arachnida, as well as from a wide assortment of insect species in the following orders: Homoptera, Coleoptera, Hemiptera, Diptera, Hymenoptera, Orthoptera, and Lepidoptera. There are also numerous references to the possible importance of this group as a factor in the natural control of harmful insects (1, 9, 10, 13, 18, 29).

New strains of *Hirsutella* have been isolated here from naturally infected larvae of the spruce budworm (*Choristoneura fumiferana* (Clem.)) collected throughout Ontario and Quebec; from larvae of the codling moth (*Carpocapsa pomonella* (L.)) collected at Simcoe, Ontario (32); and from larvae of the European pine shoot moth (*Rhyacionia buoliana* (Schiff.)) collected at Elmira, Ontario.

In infected insects, the organism forms a pseudosclerotium from which may arise numerous synnemata. These consist of compact bundles of more or less parallel, longitudinally septate hyphae, cylindric to filiform in shape, simple or branched, and narrowing toward the apex. These in turn give rise to sporogenous cells, hyaline, inflated at the base, and gradually narrowing into long slender sterigmata. Spores, borne at the apex of the slender filaments, are oblong to fusiform, one-celled, and hyaline, occurring singly or in groups of two or more within a mucus droplet.

The various experiments to be described were conducted with the spruce budworm "isolate", tentatively identified as *Hirsutella gigantea* Petch by Mains (34). The fungus is characterized by a white, later yellowish-red (YR 4/4, Munsell (38)), mycelium that gives rise to a colony with a rather tough or leathery texture on Sabouraud maltose agar (Fig. 7). Synnemata,

while frequently associated with infected spruce budworm larvae, particularly those that appear to have overwintered in a mummified state (Fig. 6), do not develop readily on artificial media. The fungus has not been observed in a sporulating state on its natural host, and is generally sterile when transferred to any of a large number of standard artificial media. Stock cultures used in these experiments were maintained by repeated transfer to Sabouraud maltose agar slants stored at 3° C.

II. General Procedure

A standard experimental procedure was adopted and used throughout this study.

The culture vessels used were "Pyrex" Erlenmeyer flasks. The glassware, first cleaned with chromic acid, was then washed in hot running tap water, single-distilled water, rinsed in several changes of double-distilled water, and subsequently drained dry. Detergents reported to have a stimulating effect on growth (35) were seldom used and then only before acid cleaning. In the initial experiments, 250-ml Erlenmeyer flasks were used; these were later replaced by smaller flasks (125 ml) of the same kind.

Each flask was stoppered with a non-absorbent cotton plug wrapped in a single layer of cheesecloth. The plugs, although handmade, were uniform in size and contained approximately the same amount of cotton. They were rolled to fit loosely into the flasks, so that they would not interfere unduly with the supply of oxygen.

All artificial or synthetic media contained salts (reagent grade) in the following concentration (in grams per liter of medium): KH_2PO_4, 1.5; $MgSO_4.7H_2O$, 0.5; $CaCl_2$, 0.01; together with the following micronutrients (in milligrams per liter of medium): H_3BO_3, 0.029; $MnSO_4.4H_2O$, 0.041; $Na_2MoO_4.2H_2O$, 0.025; $CuSO_4.5H_2O$, 0.079; $ZnSO_4.7H_20$, 0.396; $FeCl_3.6H_2O$, 0.484; $CoCl_2.6H_20$, 0.404. Approximately 0.02 g NaCl was added to each 50 ml of medium with the inoculum, which had been prepared in physiological salt solution. The amount of dextrose is specified for each experiment, since sugar is treated as an experimental variable in some of the tests. All media were prepared with double-distilled water from an all-glass still.

Hydrogen ion concentration was measured with a Beckman pH meter, Model G, equipped with a glass electrode. Before sterilization all media were adjusted with 3 *N* HCl, or 3 *N* NaOH, to pH 6, the optimum for growth.

The various preparations tested were sterilized using Seitz filters, unless otherwise indicated. While it has been shown (30) that the asbestos pads used in Seitz filters may adsorb active compounds, the resulting changes are not believed to be as serious as those that may occur within a medium as a result of autoclaving (3, 20, 47).

After sterilization, the media were transferred aseptically to the sterile experimental flasks. The volume of medium was standardized at 98 ml and 48 ml, in 250-ml and 125-ml flasks, respectively. The quantity of medium was later increased to 100 ml and 50 ml, respectively, by the addition of the inoculum in 2 ml of saline.

III. Preparation of Inoculum

The inoculum was prepared from a shaken culture grown for 8–10 days in yeast extract (1.5%) – dextrose (2%) medium. Following the incubation period it was necessary, because of the presence of a very viscous polysaccharide (31) in the medium, to add 150 ml of sterile saline to the culture. This procedure reduced the viscosity of the medium sufficiently to allow separation of the mycelium from the medium by centrifugation at 3200 r.p.m. for 20 minutes in an International type S B centrifuge. Following centrifugation the decanted culture fluid was replaced by 100 ml of fresh saline solution and the mycelial mixture was homogenized in a Waring blender at 3° C for 1 minute, centrifuged again, and then washed with saline (at least 5 times) by centrifugation (2900 r.p.m. for 10 minutes). The fragmented mycelia were homogenized again after the final suspension in saline.

The prepared inoculum, stored at 12° C, was always used within a 24-hour period. The dry weight of fragmented mycelium was determined as the mean of three 5-ml samples of the stock solution. The concentrated mycelial suspension was then diluted to give the required quantity of inoculum in a 2-ml portion. For experiments requiring a range of inoculum levels, serial dilutions were prepared.

Immediately after seeding, the cultures were placed on a rotary-action flask shaker. The shaker rotated at 260 r.p.m. describing a 1.5-in. circle and maintaining a very thin film of medium along the sides of the flask. The shaker was kept in a constant-temperature room at 23° C under continuous illumination provided by an incandescent filament bulb. The light source illuminated the surface of the shaker with an intensity of 18 ft-c.

IV. Method of Assessing Growth

The method adopted involved growing the fungus in liquid media for a defined period, weighing, and comparing the weight in the various experiments. The incubation period is specified for each experiment, since the period of growth was not always the same. Following the incubation period, the mycelium was separated from the viscous culture fluid by centrifugation in a Servall centrifuge at 10,000 r.p.m. for 2 minutes. After the supernatant was decanted, the mycelial contents were admixed with distilled water and collected on tared circles of Whatman filter paper No. 50 in a Büchner funnel (17.0 cm) by vacuum filtration after several washings. The retained mycelium was subsequently dried for 24 hours at 90° C. Each test was carried out in triplicate, the reported result being the mean of the three determinations. The design of all the experiments was such that an analysis of variance could be made.

Experimental Results

An exploratory experiment showed (Table I) that a basal medium with KNO_3 or NH_4NO_3 as the nitrogen source is not utilized by the *Hirsutella* strain. Growth, however, is distinctly enhanced when yeast extract, tryptone, or liver fraction "L" is added as a supplement. The increased growth in an enriched medium suggests that the natural products either contain some

TABLE I

Effect of natural supplements (1%) on growth response of *H. gigantea* in a basal medium with dextrose (1%) after incubation for 10 days

Supplements	Method of sterilization	Yield (mean dry wt., mg/50 ml)
Potassium nitrate* (Merck)†	Seitz filter	5.5‡
Ammonium nitrate* (Merck)	Seitz filter	4.4‡
Yeast extract (Difco)†	Autoclave (added aseptically)	270.7
Yeast extract (Difco)	Seitz filter	319.7
Autolyzed yeast extract (Difco)	Autoclave (added aseptically)	86.0
Brewers' yeast powder (GBI)†	Autoclave (added aseptically)	83.6
Tryptone (Difco)	Seitz filter	395.8
Liver fraction "L" (NBCo.)†	Seitz filter	299.4
Liver extract concentrate 1:20 (NBCo.)	Autoclave (added aseptically)	50.5

*KNO_3 and NH_4NO_3 added to provide 50 mg N/50 ml of medium.
†Merck = Merck & Co. Ltd.; Difco = Difco Laboratories, Inc.; GBI = General Biochemicals, Inc.; NBCo. = Nutritional Biochemicals Corp.
‡Growth negligible; equal weight often determined with uninoculated media on tared filter circles.

growth factor (organic or inorganic) or simply provide a more nearly optimal proportion of nitrogen and carbon components required by this organism for normal development. In order that a clearer insight into the factors concerned might be gained, a more comprehensive study of the fungus in a yeast extract – dextrose medium was undertaken, and at the same time attempts were made to develop a chemically defined medium that would support growth. By making substitutions for each of the components in the synthetic preparation, a comparative survey of the nitrogen, carbon, and growth-factor requirements of this organism would be possible. The analysis of the yeast extract product used was kindly supplied by the manufacturers.[4] In the present paper only the results as they refer to the yeast extract – dextrose medium (enriched medium) will be reported. The results of nitrogen, carbon, and growth-factor requirement studies will be presented in subsequent papers.

I. Growth in Bacto-Yeast Extract – Dextrose Medium

A. Variation Between and Within Experiments

Taber and Vining (56), in a nutritional study of three strains of *Claviceps purpurea* (Fr.) Tul., found that results obtained from repetitions of an experiment varied significantly, and for that reason only data obtained from simultaneously conducted experiments were considered for statistical comparison.

Hacskaylo *et al.* (24) reported that the results for each fungus after a given time of incubation were obtained in single experiments, which should rule out variation in the basal medium, age and vigor of the inoculum, and chance variation in the external conditions.

[4]Difco Laboratories, Detroit 1, Michigan, U.S.A.

TABLE II

Experimental data to show reproducibility in total yield between tests and between experiments (dry weight per flask culture (g); incubation period, 11 days)

		Test 1									Test 2								
		Inoculum levels (mg/50 ml)									Inoculum levels (mg/50 ml)								
	Replicates	.05	0.1	0.2	0.4	0.8	1.6	3.2	6.4	12.8	.05	0.1	0.2	0.4	0.8	1.6	3.2	6.4	12.8
Expt. I	*a*	.1785	.3551	.3362	.3330	.2690	.2961	.2856	.2879	.2775	.1612	.3458	.3470	.3329	.3097	.3168	.3141	.2825	.2691
	b	.2700	.3038	.3238	.3301	.3109	.3023	.2931	.2909	.1958	.2343	.3434	.3399	.3470	.3184	.3203	.3064	.2945	.2789
	c	.2779	.3545	.3350	.3419	.3162	.3004	.2998	.2742	.2812	.2531	.3178	.3508	.3424	.3271	.3150	.3111	.2838	.2709
Expt. II	*a*	.1862	.2401	.3500	.3482	.3180	.3070	.2798	.2818	.2630	.3180	.3090	.3294	.3310	.3162	.2969	.2605	.2354	.2400
	b	.1965	.2402	.3390	.3753	.3318	.3050	.2720	.2607	.2620	.1384	.3097	.3109	.3090	.3304	.2937	.2686	.2388	.2310
	c	.1940	.2220	.3380	.3482	.3385	.3177	.2821	.2641	.2320	.3355	.2831	.3106	.3230	.3465	.2620	.2702	.2394	.2420

Analysis of variance

Source of variation	D.F.	M.S.†	*F*
A. Experiments	1	.007211	10.8**
B. Tests within experiments	2	.000424	
C. Inoculum levels	8	.017750	26.5**
D. Inoculum levels × experiments	8	.000627	
E. Inoculum levels × tests within experiments	16	.001437	2.15*
F. Replicates or flasks treated alike (error)	72	.000669	

*Significant at 5% level.
**Significant at 1% level.
†All mean squares were tested against *F*.

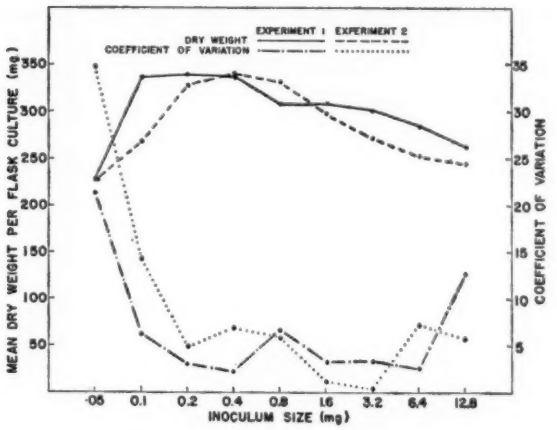
EXPERIMENT 1 EXPERIMENT 2
DRY WEIGHT
COEFFICIENT OF VARIATION
MEAN DRY WEIGHT PER FLASK CULTURE (mg)
COEFFICIENT OF VARIATION
INOCULUM SIZE (mg)
.05 0.1 0.2 0.4 0.8 1.6 3.2 6.4 12.8

FIG. 1. The effect of inoculum size on total growth (11 days) and on variation within replicates among inoculum levels, in experiments I and II. Each experiment consists of two tests (each with three replicates) run simultaneously. Inoculum size (mg) refers to dry weight of fragmented mycelium.

Pelletier and Keitt (41), in order to secure results with greater meaning, in their study of amino acids as sources of nitrogen for *Venturia inaequalis* (Cke.) Wint., ran only one replicate at one time; consequently, each replicate came from a different batch of basal medium and amino acid solutions, and a different spore suspension. The final experiment was a factorial one including 432 treatment combinations with three replicates each.

In the present study, two experiments were conducted at different times to determine if significant differences develop between separate experiments. Each experiment consisted of two tests, each test comprising nine inoculum levels as the principal variable. Each level was replicated three times within each test. The medium and parent inoculum suspension for each test were prepared separately. Experiment II was initiated 3 months after experiment I was completed, while the tests within each experiment were conducted simultaneously. The results of the study are presented in Table II.

The analysis of variance shows that there was a statistically significant difference ($P < .01$) between repeated experiments. The average difference,[5] however, was only 0.016 ± 0.005 g per flask culture. This difference between experiments, while it is statistically significant, is not large enough to be of any practical significance. Significant differences between tests within experiments occurred only in experiment II at the 0.05 and 0.10 inoculum levels.

The data in Table II also reveal that uniform yields can be obtained among replicates, particularly when the organism is grown in an enriched medium.

[5] Mean difference between experiments $= \frac{16.2549 - 15.3724}{54} = \frac{0.8825}{54} = 0.016.$

S. E. of this difference $= \sqrt{\frac{2(S^2)}{54}} = \sqrt{\frac{.000665}{27}} = .00496.$

When varied results do occur, they are largely because of the use of small inocula, 0.1 mg/50 ml or less (Fig. 1). It is believed that the extreme variation is due to the difficulty in obtaining uniform amounts of dispersed mycelium in such dilute suspensions.

B. Effect of Varying Inoculum Levels on Yield

The results given in Table II show that the inoculum level differences were significant ($P < .01$) even though they varied somewhat from test to test in experiment II (see above). It is important, therefore, that all experimental flasks within treatments are inoculated with a standard or uniform quantity of inoculum.

Maximum yields were produced by inoculum levels of 0.2 and 0.4 mg/50 ml during the prescribed incubation period (Fig. 2). Similar yields also occurred at the 0.1 and the 0.8 mg levels in experiments I and II respectively. In the 0.05 mg range the total yield was definitely lower, but would probably eventually reach a maximum with a longer incubation period (Fig. 3). An increase in levels above 0.8 mg was accompanied by a gradual decrease in yield. This decrease was partly due to the actively growing fungus cultures reaching maximum growth quickly under favorable conditions followed by a decline (autolysis). Such cultures may start with a greatly reduced lag, and with virtually no period of lag when inoculated with 12.8 mg of inoculum as shown in Fig. 3.

Whereas there was very rapid growth with large inocula, the final yield was not as great as that obtained at lower inoculum levels. The reason for this is not clear.

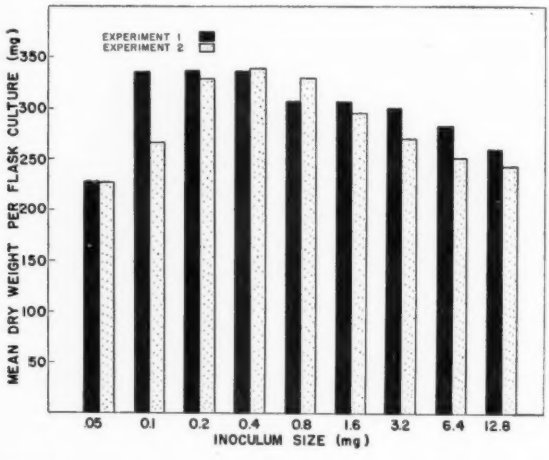

FIG. 2. The effect of inoculum size on yield within separate experiments. The larger difference at the 0.1 mg level (experiment I) can reasonably be ascribed to chance, as indicated by the nonsignificant "Levels × experiments" interaction (Table II). Incubation period, 11 days.

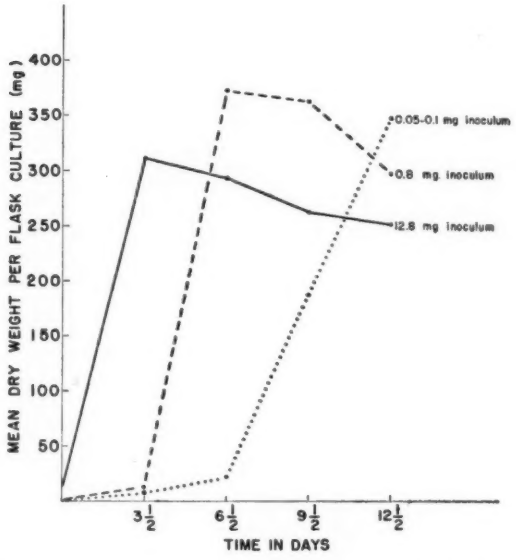

FIG. 3. Effect of varying amounts of inoculum on length of lag period and yield, in a yeast extract (1%) – dextrose (1%) medium, at intervals of 3 days over a 12½-day period. Note that there is less growth with the highest inoculum level despite the absence of a lag period.

C. Growth Response to Varying Yeast Extract – Dextrose Concentrations

A factorial experiment was conducted in which yeast extract was supplied at 0.44, 0.88, 1.5, and 2.5%, and dextrose at 1, 2, 3, and 6%. Sixteen possible combinations were set up each in three replicates, giving a total of 48 cultures in the experiment. The results are depicted in Fig. 4, while the experimental data and the analysis of variance are given in Table III.

Yields increased with an increase in yeast extract concentration up to 1.5%; thereafter yeast extract had an inhibitory effect, and at 2.5% very little growth occurred throughout the 12-day incubation period. The optimum dextrose concentration decreased as yeast extract was increased above the optimum or 1.5% level. With high dextrose concentrations at the optimum yeast extract limit (1.5%), the extracellular polysaccharides produced by this organism were extremely viscous and in some instances formed a gel-like disk, intermixed with mycelium at the bottom of the flask. These substances may have contributed to the high yield or peak (Fig. 4) obtained with yeast extract (1.5%) and dextrose (2%), although the mycelium was carefully washed when harvested. Since, however, essentially the same results developed in a repeat experiment, it was concluded that the optimum amount of yeast extract and dextrose for the growth of *H. gigantea* was about 1.5 and 2% respectively.

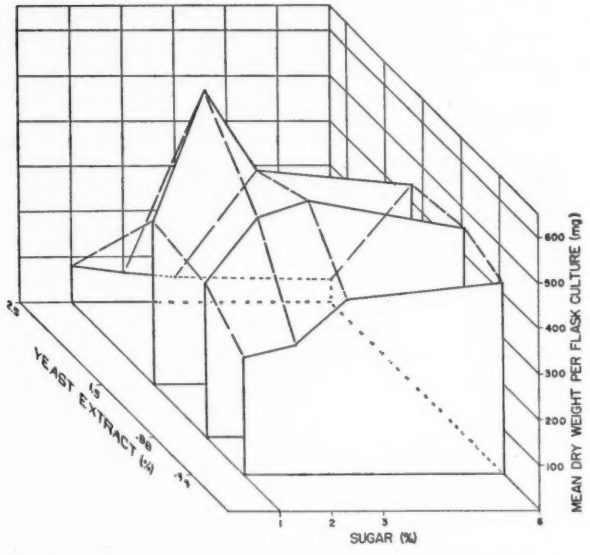

FIG. 4. Comparative yields of *H. gigantea* in a basal medium with varying concentrations of yeast extract and with different dextrose concentrations, following a 12-day incubation period. Inoculum level, 0.2 mg.

It has been found that when the polysaccharides were precipitated with ethanol or acetone, over 234 mg (dried state) of this viscous material were frequently produced in 100 ml of yeast extract – dextrose medium. Foaming was observed in several of the flasks that had produced large amounts of the polysaccharides, but the reason for the foaming is not known.

Some of the yeast extract – dextrose combinations shifted the original pH (6.0) towards acidity (down to 5.5), others in the opposite direction (up to 6.6), and still others had little effect. While maximum yield is obtained at pH 6.0, growth, however, is still relatively good at pH 5.5 and 6.5.

A second experiment was designed to test the interaction of concentration × incubation periods for yeast extract at the above concentrations (0.44, 0.88, 1.5, and 2.5%). Dextrose was added at the concentration (2%) that gave a normal mycelium with the highest dry weight of fungus in the first test (Fig. 4). The method of experimentation was exactly as in the previous experiment. The results are depicted in Fig. 5; the experimental data and analysis of variance are shown in Table IV.

The time–growth curves (Fig. 5) show that an increase in yeast extract concentration was accompanied by an increase in lag period. An incubation period of about 12 days was necessary to obtain maximum yields with the various yeast extract concentrations, with the exception of the highest level (2.5%). During this period very little growth occurred in the 2.5% concentration as shown in Figs. 4 and 5. However, following this period growth began,

TABLE III

The effect of varying yeast extract – dextrose concentrations on the growth of *H. gigantea* (Incubation period, 12 days; inoculum level, 0.2 mg; yield, dry weight per flask culture (g))

Replicates	Yeast extract .44% Dextrose 1%	.44% 2%	.44% 3%	.44% 6%	.88% 1%	.88% 2%	.88% 3%	.88% 6%	1.5% 1%	1.5% 2%	1.5% 3%	1.5% 6%	2.5% 1%	2.5% 2%	2.5% 3%	2.5% 6%
a	.2507	.2600	.4484	.5530	.3571	.5135	.5081	.4350	.2823	.6808	.2970	.4174	.0938	.1026	.0305	.0553
b	.2514	.2508	.3017	.2413	.3287	.4562	.5250	.3735	.3918	.6497	.4683	.4570	.0924	.0122	.0904	.0440
c	.2696	.3413	.4150	.4638	.3373	.4838	.5166	.5645	.4052	.6199	.6395	.4372	.0521	.0743	.0504	.0405

Analysis of variance

Source of variation	D.F.	Variance	*F*
Between yeast extract concentrations	3	0.4346	83.6**
Between dextrose concentrations	3	0.0299	5.7**
Interaction between yeast extract and dextrose	9	0.0174	3.3**
Within subgroups (error)	32	0.0052	

**Significant at the 1% level.

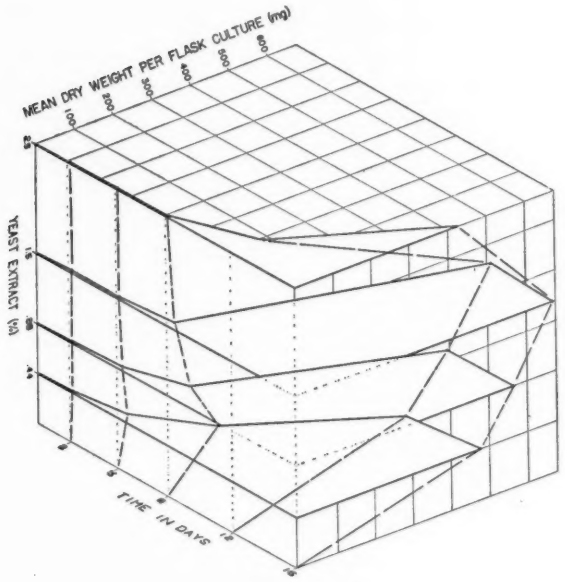

FIG. 5. Time–effect curves to show the influence of yeast extract concentrations in a basal medium with dextrose (2%) and time of incubation on yield by *H. gigantea*. Inoculum level, 0.2 mg.

and by the end of 16 days there was a substantial yield (Fig. 5). Thus it would appear that yeast extract has an inhibitory effect during the early stages of growth of this fungus.

D. Morphology of Shaken Liquid Cultures of H. gigantea

It is, of course, not new to grow fungi in shaken cultures (8, 12, 19, 39, 41), but the method appears not to have been tried before with entomogenous forms.

This species grew abundantly as small disks (Fig. 10) of relatively uniform size (Table V). In addition to the growth as disks, the cultures developed a band around the inside of each flask at the edge of the surface wetted by the medium when the flask was being shaken. This band presented the appearance of growth commonly obtained on agar media. It was fluffy, white (later turning brown), and composed of vegetative mycelium loosely intertwined, in contrast to the small disks, which were composed of compact mycelium (a central core) from which hyphae radiated out in various directions (Fig. 9).

The disks or fungal pellets develop from the mycelial fragments or minute fungal clumps added to the culture flasks as inoculum (Fig. 8). With small amounts of inoculum each of the relatively few viable particles develops into a large individual colony usually spherical (8) and somewhat variable in size

TABLE IV

Growth response of *H. gigantea* to varying concentrations of yeast extract in a basal medium with dextrose (2%) during different incubation periods
(Inoculum level, 0.2 mg; yield, dry weight per flask culture (g))

Replicates	Yeast extract																			
	.44%					.88%					1.5%					2.5%				
	Days																			
	2	5	8	12	16	2	5	8	12	16	2	5	8	12	16	2	5	8	12	16
a	.0030	.0320	.1320	.4520	.4757	.0014	.0117	.0560	.5821	.5720	.0025	.0078	.0210	.7002	.6676	.0016	.0053	.0034	.1653	.7942
b	.0038	.0248	.1490	.4622	.4881	.0042	.0125	.0641	.5440	.5643	.0037	.0063	.0183	.6820	.6631	.0027	.0054	.0050	.0518	.1970
c	.0023	.0175	.1151	.4430	.4723	.0029	.0101	.0601	.5631	.5565	.0031	.0074	.0197	.6380	.6604	.0020	.0059	.0050	.0393	.2585

Analysis of variance

Source of variation	D.F.	Variance	*F*
Between yeast extract concentrations	3	0.0824	14.5**
Between incubation intervals	4	0.7915	138.9**
Interaction between yeast extract and incubation intervals	12	0.0394	6.9**
Within subgroups (error)	40	0.0057	

**Significant at the 1% level.

TABLE V

Effect of inoculum levels on shape and size of fungal pellets grown in a shaken yeast extract – dextrose medium (sample size, 100 pellets)

Inoculum levels	Shape	Dimensions, mm	
		Range	Average
1.6% (Fig. 11, left tube)	Mostly spherical	1–6	2.6
0.8% (Fig. 11, center tube)	Mostly spherical	3–5	3.9
0.4%; typical "disk-like" appearance of culture used as source of inoculum (Fig. 10)	Mostly globoid	1.0–7.0×1.0–4.0	4.3×2.3
Low inoculum level (Fig. 11, right tube)	Mostly globoid	2–13×1–10	6.8×4.7

(Table V). According to Foster (19) this type of growth arises when competition for the available nutrients is not great among the cells present, so that each particle of inoculum has sufficient nutrient to build a relatively massive colony (Fig. 11, right tube).

When a large number of focal points are introduced as inoculum, the viable clumps develop simultaneously, forming an indefinitely large number of small compact pellets more or less comparable in size (Table V; Fig. 11, left tube).

The radiating hyphae along the outer zone or periphery of the fungal pellets, particularly with the smaller spheres, were white but the central core always displayed a faint yellowish tinge. The larger spheres were completely discolored or yellowish-brown throughout (close to YR 6/5, Munsell (38)), and imparted a yellowish tinge to the surrounding liquid. The discoloration was probably brought about by the inability of the central portion of the colonies (because of their compact nature and the enveloping polysaccharides) to secure sufficient oxygen and nutrients. Such discolored colonies were always discarded as a source of inoculum in favor of the small, white, and more viable appearing disk-like forms (Fig. 10).

When portions of mature culture containing mycelial "disks" were used as a source of inoculum, it was found that a "mycelial type" strain with very few fungal pellets developed following successive subculturing. Cultures sown with inoculum prepared from this strain were not only retarded in growth, but also produced lower total yields with low inoculum levels. The "mycelial type" strain was therefore discarded as an inoculum source. This material when washed was not unlike prepared inoculum in appearance (Fig. 8).

Discussion

Little information is available regarding the factors essential for growth of parasitic fungi attacking insects. Attempts, however, to cultivate these forms on artificial media are desirable, for success in this endeavor would ensure a ready and abundant supply of the organism in all stages of develop-

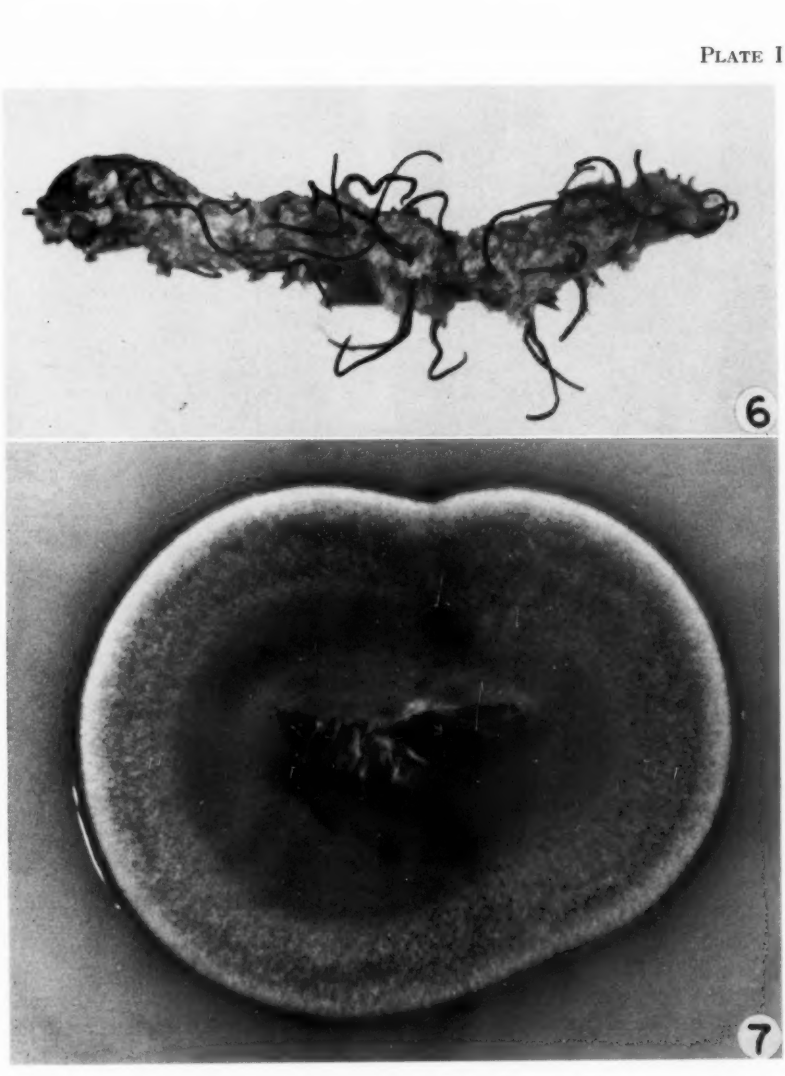

FIG. 6. A spruce budworm larva, naturally infected with *H. gigantea*, showing synnemata of varying lengths. 8×

FIG. 7. A spruce budworm pupa, naturally infected with *H. gigantea*, was surface sterilized in sodium hypochlorite, and transferred to a sterile Petri plate containing Sabouraud maltose agar. The fungus, after it appeared on the surface of the specimen, overspread the medium to form a colony with a tough mat-like surface. Note the absence of synnemata. 3×

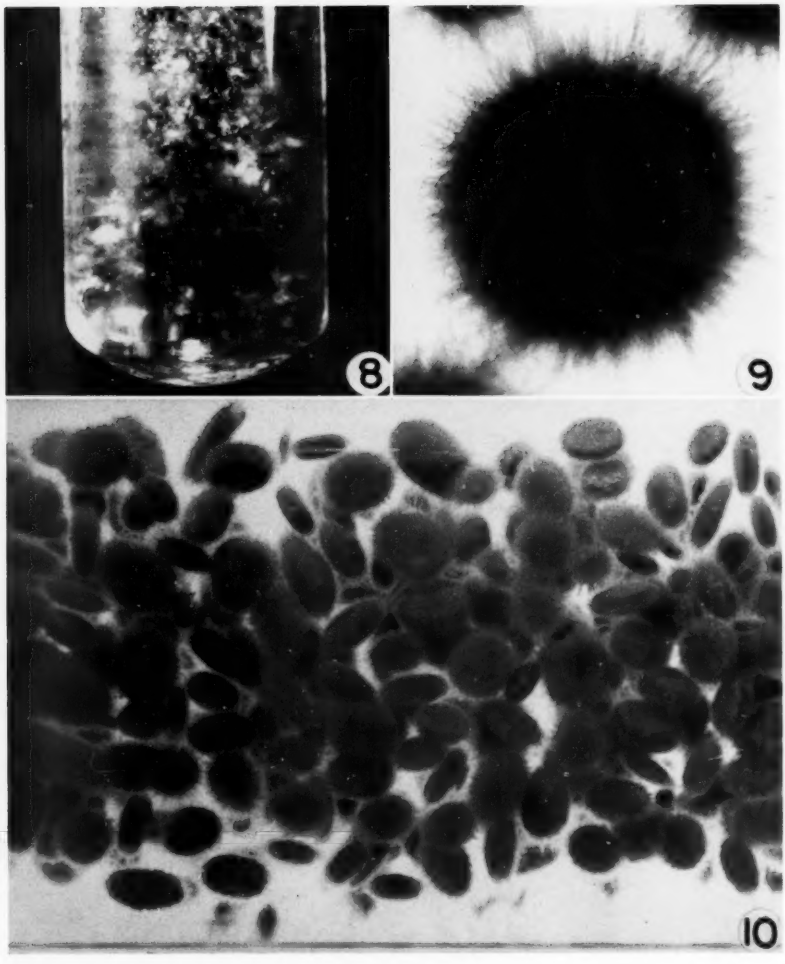

FIG. 8. Inoculum prepared from mycelial disks, after a series of washings, then fragmented in a Waring blender. Although the particles are fairly comparable in size, constant shaking of inoculum flask is necessary to maintain a uniform suspension throughout. 1.5×

FIG. 9. A spherical-shaped fungal pellet, with central core, from which hyphae radiate out in various directions. 20×

FIG. 10. Typical "disk-like" growth of *H. gigantea* in shaken yeast extract – dextrose medium. 3.2×

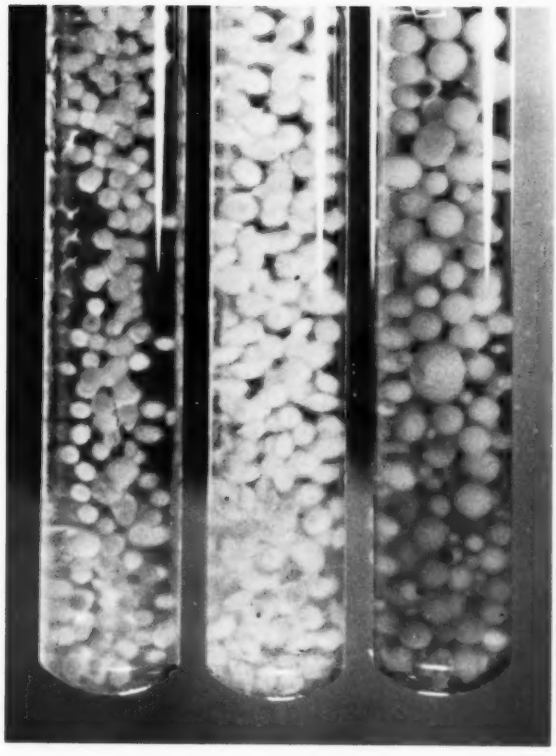

FIG. 11. The effect of varying amounts of inoculum on the shape and size of the disk-like forms produced by *H. gigantea* in yeast extract – dextrose medium (see Table V). 0.8×

ment. This would not only greatly facilitate detailed morphological study, and a more definite determination of taxonomic relationships in the group, but might also provide an important means for the control of undesirable insects, by supplying unlimited material for dissemination in the field. The genus *Hirsutella*, virtually uninvestigated with regard to nutrition and sporulation, was selected for this study because of the dense vegetative growth produced by some of its strains in natural media, and partly because of its apparent stability in slant culture. The experimental strain *H. gigantea* was isolated in pure culture on Sabouraud maltose agar from naturally infected spruce budworm larvae.

With the experimental procedures described in this paper, the growth response of *H. gigantea* was studied in a yeast extract – dextrose medium; the results to serve as a basis for a comparison of growth within a chemically defined medium (to be published in subsequent papers). Experiments conducted to determine differences between separate experiments in which varying amounts of inoculum served as the principal variable showed that the experiments were significantly different, although the average difference between experiments was only 0.016 ± 0.005 g per flask culture. This size of the difference, however, was unimportant, since it was reasonably uniform for all inoculum levels. Both experiments showed that the optimum inoculum level was from 0.2 to 0.4 mg/50 ml, and that with this amount results from repeated experiments were relatively uniform. With small quantities, 0.1 mg or less, yields were less reproducible both between and within experiments. It is believed that this variation is due in part to the difficulty in obtaining uniform amounts of dispersed mycelium in such dilute suspensions, and possibly to natural variation. On the other hand an increase in the amount of inoculum above the optimum, while accompanied by a higher growth rate, resulted in a gradual decrease in total yield. The reason for this is not clear; it is suggested, however, that the high growth rate might have led to inefficient utilization of the nutrients and/or to a more rapid accumulation of inhibitory substances. While the use of large inocula (within limits) may be important when the time factor is critical, as in the development of industrial processes (19) their use in nutritional studies must be treated with caution, since they could result in reduced yields, and moreover, conceal growth factor requirements in chemically defined media by carrying over additional nutrients with the mycelial fragments.

In experiments to determine the optimum yeast extract – dextrose concentration, it was found that the highest yield was produced with 1.5% yeast extract and 2% dextrose. At higher concentrations yeast extract appeared to have an inhibitory effect, and at 2.5% very little growth was produced by the fungus during the 12-day incubation period. Later some growth began to take place and at the end of 16 days there was a substantial yield. Thus it would appear that yeast extract has an inhibitory effect in the early stages of growth. At the same time, in view of the fact that some growth eventually takes place, the fungus must adapt itself to the high yeast extract concentration.

In the enriched shaken liquid medium, *H. gigantea* grew abundantly as small disks of relatively uniform size (4.3×2.3 mm). The morphological alterations of several fungi in submerged shaken cultures have been treated by Burkholder (8) as morphogenesis. Foster (19), however, questioned whether this phenomenon should be considered as morphogenesis, since the curious growth pattern is not a fundamental link in the biological development of the organism, but rather a manifestation of a set of peculiar physical conditions.

A basal dextrose–salts medium in which yeast extract is replaced by potassium nitrate, or ammonium nitrate, as the nitrogen source, is not utilized by this organism. The enhanced growth with yeast extract demonstrates that an organic nitrogen source is required for growth; yeast extract may, in addition, act as a source of growth factor(s).

Relevant literature on the nutritional requirements of entomogenous fungi is briefly mentioned.

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PALAEOBOTANICAL STUDY OF BURIED PEAT FROM THE MACKENZIE RIVER DELTA AREA, NORTHWEST TERRITORIES[1]

J. TERASMAE

Abstract

A palaeobotanical and palynological study of samples of buried peat from the MacKenzie River delta area, Northwest Territories, has shown that the peat accumulated during an interglacial interval. For reference purposes a study of modern pollen of *Rubus chamaemorus* L. and of four species of *Drosera* L. has been made.

Several years ago, A. E. Porsild of the National Museum of Canada observed layers of buried peat in exposures along the east branch of the MacKenzie River in the delta area. According to him the buried peat was overlain by Pleistocene deposits of considerable thickness (Fig. 1). A part of this overburden was tentatively identified as glacial till. Samples of the buried peat were collected by Porsild and a palaeobotanical and palynological study of these samples was made by the writer.

The compressed peat can be classified as a poorly decomposed, woody *Sphagnum* peat (Figs. 2 and 6). Twigs of ericaceous shrubs are abundant and leaves of *Chamaedaphne calyculata* were identified (Figs. 2 and 3). When dry the peat is platy and can be easily split along the bedding planes. A cone of *Larix laricina* (DuRoi) K. Koch (Fig. 6) and wing covers of beetles were discovered. Leaves of *Ledum* and stems of *Oxycoccus microcarpus* Turcz. were identified in some samples of the peat.

Samples for the palynological study were first boiled in dilute solution of potassium hydroxide, then screened to remove the coarse plant detritus and then treated with the acetolysis method (4).

Results of the palynological study have been illustrated in Table I. For pollen and spores encountered outside the pollen count or for which no percentage was calculated the following symbols have been used: + (present), ++ (abundant), +++ (very abundant).

An interpretation of the palaeobotanical and palynological data indicates that the vegetation at the time when the peat accumulated was closely similar to the modern vegetation of the area and thus also the climatic conditions then should have been comparable with the present. Hence, interglacial conditions are inferred from the study of the buried peat. Indeed, the climatic conditions during some part of the interglacial interval under consideration may have been even more favorable than the present as indicated by the discovery of the tamarack cone and the abundance of spruce pollen in some samples. Judging by the present distribution limits given for spruce and tamarack (2, 3), the northern range of *Larix laricina* and the

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Contribution from the Geological Survey of Canada, Ottawa, Canada. Published with the permission of the Director, Geological Survey of Canada.

TABLE I

Pollen types	Sample number 1	2	3	4
	Per cent of total tree pollen			
Arboreal pollen (AP)				
Picea mariana	5.5	1	8	12
Picea glauca	4.5	2.5	10	9
Pinus banksiana	5	2	11	9
Abies cf. *lasiocarpa*	1	–	–	–
Larix laricina	3	–	1	–
Alnus	49	70	45	57
Betula	31	21	22	11
Salix	1	3.5	3	2
Non-arboreal pollen				
Ericaceae	8	20	11	7
Rubus chamaemorus	–	1	1	3
Chenopodiaceae	+	–	–	–
Artemisia	2	+	2	–
Compositae	–	1	–	–
Umbelliferae	–	+	–	–
Cyperaceae	–	–	21	2
Gramineae	1	1	–	–
Myrica gale	–	+	14	13
Drosera rotundifolia	–	1	–	–
Unidentified	2	3.5	2	2
Total NAP	13	27.5	51	27
Spores and miscellaneous				
Polypodiaceae	+	–	+	–
Equisetum	+	–	–	–
Sphagnum	+++	++	+	+
Lycopodium annotinum	–	+	–	–
Fungus remains	+	++	+	+
Diatoms	–	+	–	–
Charcoal fragments	–	+	–	–
Silt and sand grains	+	–	–	–

black and white spruce may have extended north of their present known range during that interglacial interval.

A correlation of this postulated interglacial interval with the recognized Pleistocene stratigraphic column must wait until further studies have been made.

In some samples of the buried peat from the MacKenzie River delta area pollen grains of *Rubus chamaemorus* L. and *Drosera* L. were identified. In order to confirm these identifications a study was made of modern pollen of *Rubus chamaemorus* and four species of *Drosera*.

The pollen grains of *Drosera* are united in tetrads, generally tetrahedral in arrangement. Exine of the distal surface of the individual grains is thick, rigid, and provided with spines; that of the proximal surface is soft and flexible and is thrown into plaits which converge toward the center of the tetrad (1, 5). The germ pores (apertures) are found as arch-like openings between the plaits on the inner surfaces of the pollen grains.

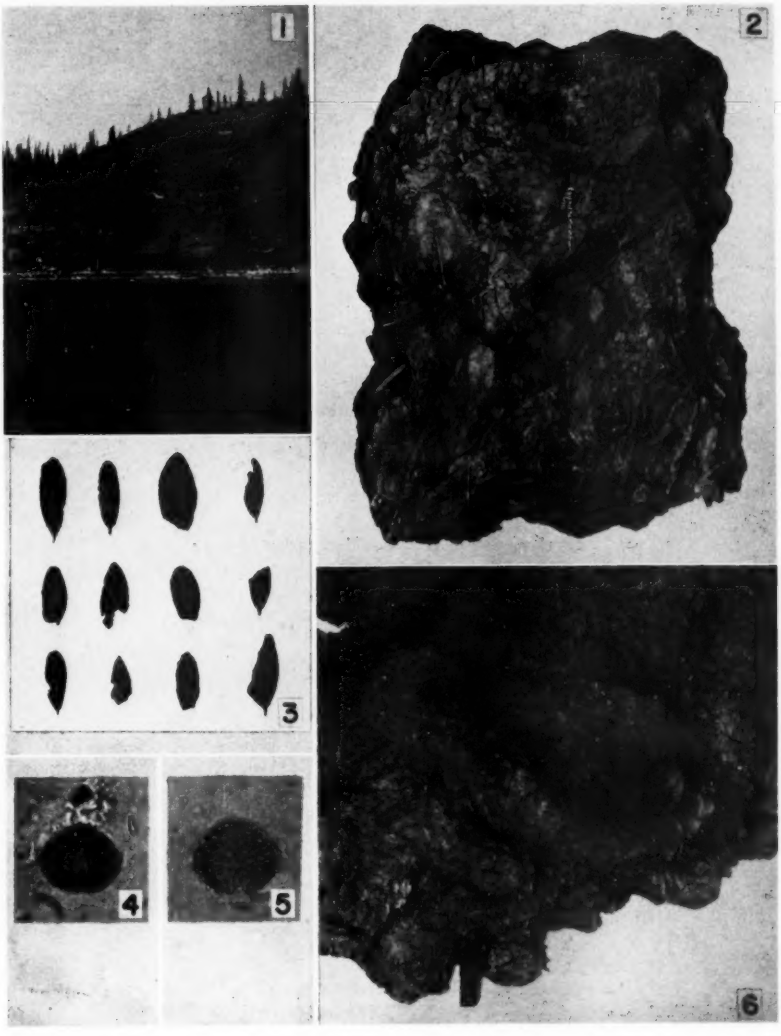
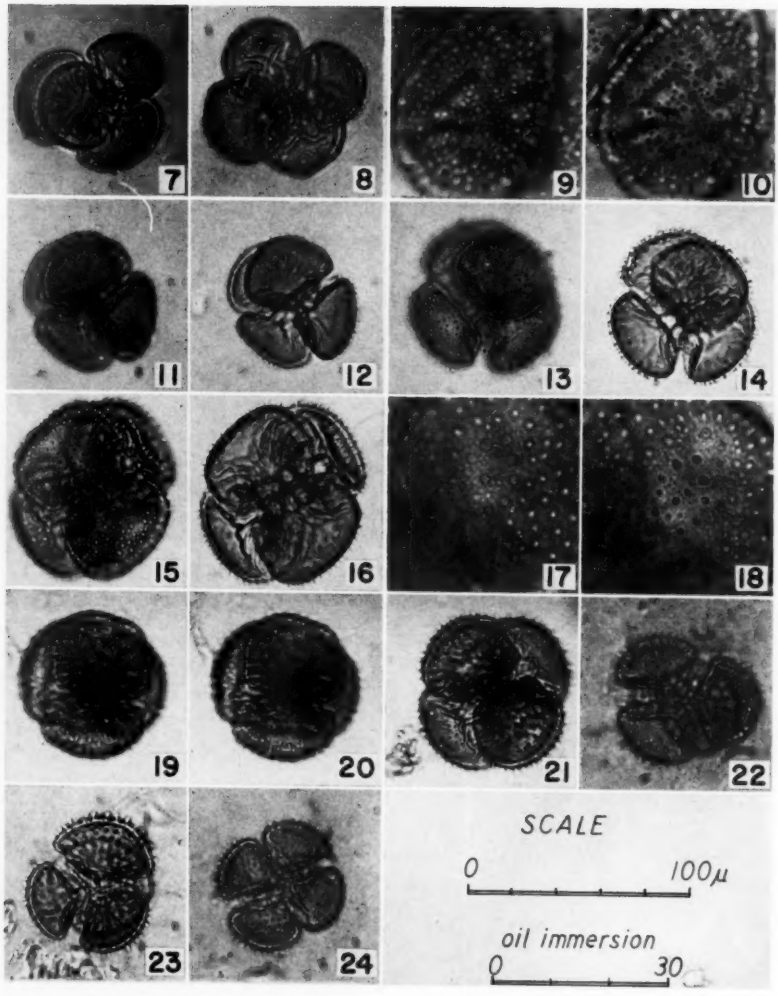


FIG. 1. General view of exposure of Pleistocene deposits on east branch of MacKenzie River in the delta area where layers of buried peat were discovered. Photo by A. E. Porsild.
FIG. 2. A sample of interglacial peat showing leaves of *Chamaedaphne calyculata*. Length of leaves 1.5–2.5 cm.
FIG. 3. Leaves of *Chamaedaphne calyculata* from interglacial peat. Length of leaf at upper left is 2.3 cm.
FIGS. 4, 5. Pollen grains of *Rubus chamaemorus*. Diameter of grains about 37 μ.
FIG. 6. Cone of *Larix laricina* in the interglacial peat. Length of the cone is 1.1 cm.



FIGS. 7–10. Pollen grains of *Drosera intermedia*. Diameter of tetrads ca. 64 μ. Figs. 9–10. Detail of exine surface showing arrangement of spines under high and low focusing (oil immersion).
FIGS. 11–14. *Drosera rotundifolia* f. *breviscapa*. Size range of tetrads 57–69 μ.
FIGS. 15–18. *Drosera anglica*. Size of tetrads ca. 80 μ. Figs. 17–18. Details of exine surface under oil immersion.
FIGS. 19–21. *Drosera linearis*. Size of tetrads ca. 69 μ.
FIGS. 22–24. *Drosera rotundifolia*. Size of tetrads ca. 58 μ.

Drosera intermedia Hayne. Collected by H. J. Scoggan, No. 13323, from Shippigan Island, New Brunswick, August 10, 1956. Diameter of tetrads 64.5 μ, ranging from 55 to 70 μ. Individual grains roundish, loosely united in tetrads. Spines small, irregularly spaced, with blunt and slightly swelled ends (Figs. 7–10).

Drosera rotundifolia L. f. *breviscapa* (Regel) Domin. Collected by M. L. Fernald and K. M. Wiegand, No. 3496, from Belle Isle, Labrador, August 5, 1910; revised by H. J. Scoggan, 1955. Diameter of tetrads 63 μ, ranging from 57 to 69 μ. Individual grains of the tetrad flattened. Spines small, numerous, irregularly spaced with blunt ends (Figs. 11–14).

Drosera anglica Huds. Collected by Porsild and Breitung, No. 11479, from Yukon Territory, Canol Rd., Mt. Sheldon, August 4, 1944. Diameter of tetrads 80 μ, ranging from 72 to 88 μ. Tetrads round and compact. Spines numerous, irregularly spaced with blunt ends (Figs 15–16). The spines are of two sizes (Figs. 17–18).

Drosera linearis Goldie. Collected by Baldwin and Breitung, No. 3621, at Longlac, Ontario, July 30, 1952. Diameter of tetrads 69 μ, ranging from 64 to 74 μ. Tetrads round and compact. Spines of two sizes and with sharp ends. The larger ones are spaced rather regularly (Figs. 19–21).

Drosera rotundifolia L. Collected by Taylor *et al.*, No. 1230, at Corbeil Point, Algoma District, latitude 47° 00′ north and longitude 84° 45′ west, July 22, 1935. Diameter of tetrads 58 μ, ranging from 53 to 64 μ. Individual grains loosely arranged in the tetrad. Spines small, irregularly spaced with both blunt and pointed ends (Figs. 22–24).

Rubus chamaemorus L. Collected by W. K. W. Baldwin, No. 4784, at Iroquois Falls, Ontario, June 15, 1953. Pollen grains 3-colpate, subspheroidal, diameter about 37 μ, ranging from 34 to 43 μ. The surface of the pollen grains is provided with blunt spines, spaced irregularly and varying in length (Figs. 4–5).

The study of modern pollen confirms the identification of *Drosera rotundifolia* L. pollen in samples of the buried peat. Pollen of *Rubus chamaemorus* and *Drosera rotundifolia* has been found in several samples of interstadial and postglacial peat from eastern Canada.

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THE GERMINATION OF LIGHT-SENSITIVE SEEDS OF TYPHA LATIFOLIA L.[1]

H. B. SIFTON

Abstract

Germination in *Typha latifolia* L. is strongly favored by white light and by low oxygen concentration. Microscopic study shows that the effect is on the aleurone grains, whose colloids are so affected that they attract and hold water more strongly, and the resultant swelling ruptures the seed coat. The swelling of the grains was observed under various sets of conditions. Its progress is described and has a bearing on current theories with respect to these structures. The process of vacuolation in the cells was also studied. On it light has no apparent effect, but it is notably stimulated under conditions of vigorous, aerobic respiration. In some samples of seed, where the tendency to vacuolate is especially strong, atmospheric pressure of oxygen strengthens vacuolation to an extent that will compensate for its depressing effect on swelling of the grains, and satisfactory germination follows. In other samples this is not so. Under very low light intensity, rather wide alternations of temperature promote germination. This was found to be due to promotion of vacuolation. Respiration studies show slow respiration and a low respiratory quotient at the low temperature. It is suggested that this allows a clearing away, during the time at low temperature, of the products of respiration, thus allowing, when the higher temperature is resumed, the vigorous respiratory activity that makes for vigorous vacuolation.

Introduction

In 1926, Morinaga published two papers (32, 33) on seed germination in which seeds of *Typha* were mentioned, and his findings suggested the present investigation. He reported that *Typha* seeds gave a much higher percentage of germination under water or with reduced oxygen pressure than in air; that their germination was greatly favored by light; that "to obtain best germination of intact seeds, one or more of these conditions must be combined with alternating temperatures"; but that if the coats were broken, the above-mentioned conditions became unnecessary. A species with such a combination of characteristics seemed to be promising material for further research.

The investigations now to be reported were carried on more or less sporadically over a period of years, and as a result some data have emerged, in addition to what might have been observed had the work been done more expeditiously.

Typha seeds are convenient objects for investigation. The several thousand seeds of a single inflorescence are remarkably consistent in their reaction, and sampling difficulties are thus at a minimum. Moreover, the seeds germinate readily and promptly, and their small size provides for economy of space in the germination chamber, and is desirable also in experiments where units of weight are used, and a large number of seeds per unit weight is an advantage.

Environmental Factors and Germination

In Morinaga's work, *Typha* was merely one of a number of species on which germination tests were made. A more intensive investigation along similar

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Contribution from the Department of Botany, University of Toronto, Toronto, Ontario.

lines has been productive of additional pertinent details. Cubical water baths, 2 ft in diameter, were used for the experiments. The temperature was thermostatically controlled to well within one-tenth of a centigrade degree, as indicated in routine readings made twice daily. Light was supplied by a 150-w, 120-v light bulb in a cylindrical container of pyrex glass, sunken below the water surface in the center of each tank.

After some experimenting it became standard procedure to use as seed containers screw-topped vials, $3\frac{1}{2}$ in. long and $\frac{7}{8}$ in. in diameter. These were found to be quite adequate as germination chambers for samples of 50 or 100 of these small seeds, either immersed in water (20 cc per vial) or spread on moist strips of blotter or filter paper in air or gas mixtures. The vials, attached to sheets of wire mesh, were suspended in the water of the baths, at a distance of about nine inches from the lights. It was not practicable to have all the samples at exactly the same distance, but the experiments indicated that the slight differences in illumination were not responsible for significant differences in germination.

Effect of Temperature

The most suitable constant temperatures for germination are rather high, from 25° C to 30° C. For one representative sample, the percentages of germination in light at various temperatures are shown in Table I. Each result is the average of 10 tests of 50 seeds each. At 15° C germination is very slow, and 20 days were allowed for the test. For the higher temperatures the 10 days allowed were more than sufficient.

TABLE I

Germination in light at constant temperatures

	% germination	
Temperature	Immersed in water	Moist blotters in air
35° C	81	48
30° C	89	61
25° C	86	44
20° C	69	37
15° C	24	20

A temperature of 30° C is clearly satisfactory for the germination of these seeds when other conditions are right. A temperature of 35° C is somewhat too high, and 20° C is clearly too low. The optimum is probably somewhere between 25° and 30°, and nearer the higher temperature. This fact was clearly indicated throughout the investigation.

Effect of Oxygen Pressure

The results shown in Table I agree with those of Morinaga in that seeds immersed in water invariably gave a higher germination count than those on moist blotters in air, and this is clearly due, as he thought, to the low oxygen

TABLE II

Germination in light at 30° C at reduced oxygen concentration

Conditions of test	% germination				
	Sample No.				
	1	2	3	4	5
Immersed in water	94	100	96	75	99
On moist blotters in 2% oxygen	98	100	99	90	100

supply of the submerged seed. This is confirmed by Table II, which gives comparative results for five samples, from the crops of 4 different years.

The rather better results with the gas mixture were probably due to the more rapid diffusion in this medium. The experiments and observations of the present investigation give no evidence that a greater leaching of useful materials from the immersed seeds was a factor.

Many germination tests were made to establish the quality of material used in the latter part of the work, and these established the fact that with some samples reduced oxygen pressure was not essential to optimum germination. A series of such tests, extending over 7 years, is recorded in Table III. From the crops of 4 of the 7 years, most of the samples tested gave much higher germination counts under reduced oxygen pressure, but 3 of these 4 years produced one sample each in which little or no difference was apparent. In the other 3 years the samples tested showed no significant advantage of one method over the other. This difference in response provides an additional opening for research into underlying causes.

The literature contains reports of some other species of seeds whose germination is favored by reduced oxygen pressure. Morinaga (33) named *Cynodon dactylon* (L.) Pers., *Roripa nasturtium* Rusby, and *Trifolium repens* L. as possessing this characteristic. Concerning the light-sensitive seeds of *Lythrum*

TABLE III

Germination in light at 30° C with or without reduced oxygen concentration

Year harvested	% germination		Year harvested	% germination	
	2% oxygen	Air		2% oxygen	Air
1950	99	87	1952	85	30
	100	100		96	78
	99	82		98	91
1951	96	64		100	98
	99	33		100	84
	99	52	1953	99	98
	99	55		99	99
	100	62		99	99
	98	44		100	98
	90	39		99	99
	100	96	1954	100	97
			1955	97	73
			1956	99	99

salicaria, *Epilobium hirsutum*, *Nicotiana tabacum*, and *Elsholtzia patrini*, Böhmer (5) found that the percentage of seeds germinating in darkness decreased with an increase in oxygen pressure. Migita, Kawana, and Takahashi (31) have also something to say along similar lines, reporting that in *Pinus densiflora* S.& Z. the seed coats are broken more rapidly in germination under low oxygen pressure, and highest germination is obtained when this condition is imposed during the earlier stages, and discontinued after the coat is broken.

Effect of Light

Intensity

Seeds of *Typha* are remarkably sensitive to light. Without light, germination is very low. Table IV compares germination under water in darkness at favorable constant temperature with that in the light of a 150-w, 120-v bulb. For tests in darkness the vials containing the seeds were wrapped in two or more thicknesses of metal foil. The nine lots of seeds tested were from the crops of 6 different years.

The intensity of light necessary to bring about germination is low, and the critical intensity varies widely for individual seeds. In one experiment the germination vials were wrapped in heavy herbarium mounting paper and kept in the lighted tank at 30° C for 10 days. The tests in this case were done in quintuplicate and the vials were arranged in a circle with the light source at the center. In unwrapped vials this sample of seed germinated 99%. Wrapped in three layers of paper the germination was still 97%, but in four layers it was only 85%, in 12 layers 42%, in 18 layers 15%, and in 20 layers 10%. With all light excluded by tin-foil wrappings the germination was 5%.

Alternating Temperatures at Low Intensity

Morinaga reported satisfactory germination in darkness when certain alternations of temperature were employed, and such results were to be expected in the light of work on certain other seeds favored by light. Von Liebenberg (28) found that alternations helped greatly in the germination of *Poa*, a finding confirmed by numerous later workers. Gassner (15) obtained complete germination of afterripened seeds of *Chloris ciliata* in darkness by the use of daily alternations. He also (16) found such treatment favorable for 10 other species favored by light, and other workers (37, 22, 10, 4) have since obtained similar results.

TABLE IV

Germination in darkness and in light

Conditions of test	% germinating								
	Sample No.								
	1	2	3	4	5	6	7	8	9
In darkness	1	6	0	4	0	1	4	10	6
In light	91	99	99	100	95	95	94	97	98

In the present investigation, tests carried out in 2 successive years on current crops showed no beneficial results from the use of temperature alternations when no light reached the seed. In the first test, seed that gave a germination of 99% in light and 3% in darkness at constant temperature germinated 2% without light and with alternations of 35° C and 20° C (8 hours at the higher temperature each day). The second sample, from the next year's crop, germinated 90% in light and 12% in darkness at the constant temperature, and gave 12% also with alternations of 35° C and 15° C.

When tests were made under very low light intensity, as opposed to complete darkness, the alternations were found to have some effect, as shown by the following typical examples. The lot of seed mentioned above as giving 12% germination in darkness germinated as follows when wrapped in 12 thicknesses of mounting paper:

at 35° C constant	44%,
at 30° C constant	44%,
at 30° C and 20° C alternating	51%,
at 35° C and 15° C alternating	69%.

Comparable tests with another sample, using 18 thicknesses of the paper, resulted as follows:

at 30° C constant	59%,
at 30° C and 20° C alternating	86%,
at 35° C and 15° C alternating	83%.

In all cases the alternations, acting under much reduced light intensity, brought about germination in some of the seeds that failed to germinate at constant temperature.

Later, when it had been discovered that some samples of seed germinated very well in air, it was found that such seeds were affected by alternation of higher and lower temperature, even in complete darkness. In preliminary tests, the sample dealt with in Table V was found to germinate 100% under water and 94% in air at a temperature of 30° C in light. Table V shows the results obtained in darkness at four different alternations of temperature, and at 30° C constant temperature. At each alternation, three separate tests were made. In the first test, the vials containing the seeds were wrapped individually in aluminum foil, tied in bundles, suspended in the constant-temperature tanks, and changed from the lower temperature to the higher at 9.00 A.M. and back to the lower at 5.00 P.M. each day. At the conclusion of the test, the range of results among the 10 replicates in each case was so great that the work was repeated, with each group of wrapped vials enclosed in a wide-mouthed gallon jar, to obviate the possibility of the foil becoming perforated by handling during the experiment. Lastly, the light was excluded by enclosing the groups of vials in the boxes of tin-plate used for the storage of motion picture film. The two significant points are the considerable percentage of seeds germinating, and the wide variation in results among the 10 replicas of a single test. Of the 12 tests made, the replicate samples of one checked fairly well, namely that in the film box at alternations of 35° and

TABLE V

Germination in darkness at alternating temperatures of seed only slightly inhibited by atmospheric pressure of oxygen

Temperature	Darkening agent	% germination of individual samples										Av.
30° and 15° C	Aluminum foil	100	100	100	96	84	82	36	16	14	0	62.8
30° and 15° C	Foil in jar	96	90	78	70	62	50	24	22	12	6	51.0
30° and 15° C	Metal film box	100	96	96	94	94	88	85	82	76	—	90.1
35° and 15° C	Aluminum foil	96	94	88	86	86	84	82	74	74	70	83.4
35° and 15° C	Foil in jar	92	90	88	82	72	60	18	8	6	4	52.0
35° and 15° C	Metal film box	98	96	94	92	80	69	67	62	48	48	75.4
35° and 20° C	Aluminum foil	92	92	90	88	88	76	62	48	44	40	72.0
35° and 20° C	Foil in jar	82	76	64	24	20	14	6	6	4	4	30.0
35° and 20° C	Metal film box	100	100	100	100	98	98	98	98	94	92	97.8
30° and 20° C	Aluminum foil	100	98	96	94	94	94	90	90	86	80	92.2
30° and 20° C	Foil in jar	12	12	6	6	6	4	2	0	0	—	5.3
30° and 20° C	Metal film box	100	94	90	65	64	58	35	27	27	6	56.6
30° C	Metal film box	6	6	4	4	4	4	4	4	2	2	4.0

20° C, and on examination the cause of the high and even germination in this case was seen to be a small hole rusted through the wall of the container, admitting light.

In these seeds, there appears to be a delicate balance between favorable and unfavorable factors for germination under the conditions provided, so that very small variations in environment can shift the weight from one side to the other. Morinaga (32) was perhaps dealing with seeds of this type when he reported the efficacy of certain alternations of temperature in complete darkness. His published results show evidence of behavior similar to that recorded in Table V when his graph shows, for example, a germination of 90% at alternating temperatures of 32° and 15° C, and 2% at 27° and 15°. The behavior shown in Table V might well produce results such as his if the number of replicate tests were small.

Color of Light

When the longer wave lengths of visible light are excluded by wrapping the germination vials in blue cellophane, the germination picture resembles that produced by darkness, but with some differences. This is in agreement with the findings of numerous earlier workers with other light-sensitive seeds (18, 23, 21, 24, 12, 30, 2, 26, 19). There is a question whether the light passing through the filter acts as an inhibitor, or the blue filter merely shuts out rays that are stimulating. Recent work with light-sensitive lettuce seed has emphasized the importance of infrared as an inhibitor (6, 11, 7), and one team of workers (11) found that the inhibiting effect of blue and green filters was due to their transmission of infrared while shutting out the stimulating red rays. Whatever the cause, the germination of *Typha* seed when wrapped in blue cellophane is invariably low, as may be illustrated by two typical examples of seeds of which the germination was 98% and 95% respectively

TABLE VI

Germination at alternating temperatures with wrapping of blue cellophane. Same seed as in Table V

Temperature	% germination of individual samples										Av.
35° and 18° C	16	16	14	14	8	4	4	4	2	0	8.2
30° and 18° C	6	4	2	2	2	2	2	2	0	0	2.2

in clear light, 10% and 7% in darkness, and 8% and 7% in the blue light. Some of the so-called blue cellophane on the market transmits a considerable amount of red light, and since red light is stimulating to these seeds, such cellophane is unsuitable for use in an experiment such as this.

Under the blue filter, alternating temperatures produce little or no improvement in germination, even with seeds such as those dealt with in Table V. Two tests of 10 samples each were made from seed of that same lot, and the results are shown in Table VI. Neither the increased germination nor the wide variation in results found in the comparable experiment in darkness is evident here, though there is a slight tendency in that direction in the test with the greater alternations.

Effect of Rupture of the Seed Coat

In a number of species of light-sensitive seeds, as, indeed, in many others, the enveloping structures (integuments, pericarps, or hulls) have been found to exert a restraining influence on germination. With *Chloris ciliata* (14) and *Poa* (29, 1), germination is improved by removing the hulls. With *Epilobium hirsutum* and *Rumex crispus* (3) and with *Arabidopsis thaliana* (30), and *Lythrum salicaria* (25), the effects of darkness are said to be overcome by pricking or rupturing the coats.

In Morinaga's experiments the breaking of the coats of *Typha* seeds was accomplished by removing the small cap that covers the distal end of the seed and is in normal germination forced off by the expanding contents, leaving an aperture through which the seedling emerges. With this cap artificially removed, he obtained good germination in darkness or in air at constant temperatures. The results obtained in the present work are in agreement with his, and bring out some additional points. One sample, with seeds intact, gave a germination of 99% in light and 4% in darkness, but with the caps removed the germination in darkness was 99%, and with a needle prick in the side it was 92%. When tested in light that passed through the blue cellophane filter, this same sample with pricked seeds germinated only 10%. Another sample from an earlier year's crop gave 81% germination in unscreened light, 13% in blue light, and 31% in blue light with caps removed. The behavior of these two samples is typical. In complete darkness the inhibition of germination is overcome by removing the caps from the seeds, and to a lesser degree by pricking them in the side. The radiation passing the blue filter produces an inhibitory effect that is much less affected by these mechanical measures.

The efficacy of breaking the seed coat in facilitating germination is apparently not entirely due to the elimination of the confining pressure on the embryo. In some of the pricked seeds, abnormal emergence through the hole in the side takes place, but more often the cap is forced off by the vigorously expanding embryo, and normal germination occurs.

Observations on the Germination Process

The voluminous literature on light-sensitive seeds includes some hypotheses concerning the actual processes of germination and their relationship to light. Cieslar (8) suggested that assimilation in light might be a factor, but evidence of this is lacking, and there are no visible signs of the presence of chlorophyll at this stage. In his suggestion that light might act through a more speedy transformation of food reserves, he was supported by Heinricher (20). Lehmann and Ottenwälder (27) went a step further, suggesting a catalytic action of light on the hydrolysis of stored protein. Theories have also been advanced that germination is prevented by inhibiting substances, either present in the seed coats (35) or in surrounding mucilage (13) and having their effect destroyed by light, or developed during germination in darkness (17, 25). Axentjev (3) and Paech (34) approach the problem from another angle, emphasizing oxidation processes and the effect of light on them.

In the present work some examination of morphological developments during germination was undertaken. The seed of *Typha* is, in form, a small cylinder, drawn to a point at the chalazal end, and covered at the other by a flattened cap with a papilla at the center. The heavily suberized coat is not only waterproof, but tough. Its weakest part is in a ring about the edge of the cap. Inside the coat, a layer of starch-bearing endosperm cells surrounds the embryo, which is oriented with the tip of its radicle directly beneath the cap. During germination the cap is forced off, a clean break taking place at the ring of weaker tissue.

Aleurone Grains and Their Swelling and Dissolution

The cells of the embryo, with the exception of those of the procambium, are copiously supplied with aleurone grains, which are found to play an interesting part in the germination process. A century of study of aleurone grains has produced two schools of thought, defended in recent times by Wieler (37) and Dangeard (9) respectively, whose papers include a good bibliography of earlier work.

Wieler maintains the thesis that aleurone grains normally originate in the cytoplasm of cells, arising as a result of the coming together of solutions of substances that combine to form a precipitate. He does not exclude the possibility of their occasional appearance in vacuoles by this same process, but suggests formation in cytoplasm as the more typical course of events. He says their beginning is evident while the seed is still young, and is not due to the withdrawal of water.

Dangeard, on the other hand, considers that the grains are merely vacuoles, rich in albuminoids and progressively dried. He describes the fragmentation

of large vacuoles into smaller ones, each of which, by dehydration, will become an aleurone grain in the mature seed. In some kinds of seeds accumulation of albuminoid reserves may take place early, and the beginnings of grains may be seen, in the form of precipitated globules, before the large vacuole fragments. Dangeard watched the behavior of the grains during seed germination, and reports a complete reversal of the above process, the vacuoles being formed again by hydration, solution, and fusion of the aleurone grains.

Appearance of the Grains

Figure 1 shows a small group of the aleurone grains of a seed of *Typha*, mounted in glycerin and photographed through a bright contrast phase objective. Embedded in the ground substance of each grain, which appears white in the photograph, one or more granules can be seen, appearing dark. The number of granules per grain, and the size of individual granules, varies greatly, many of the grains containing larger numbers than those shown in the photograph. The granules appear dark also when viewed through the conventional microscope. The contrast is much more striking, however, and their actual size is more clearly seen, when the phase microscope is used.

Changes in Grains During Water Absorption

In cells of the young seedling, very soon after the seed coat has been broken, the aleurone grains are no longer to be seen, but each cell contains a single large vacuole surrounded by a thin layer of cytoplasm in which are embedded large numbers of small granules that appear bright, rather than dark. Some difficulty was experienced in seeing exactly what happens to the grains during the process of water absorption and germination. If grains from a crushed embryo are mounted in water they change very rapidly, and by the time the preparation is ready for observation one sees groups of bright granules, or often a confusing mixture of brighter and darker material, instead of the typical aleurone grains. Unsuccessful attempts to observe the process of change were made by mounting grains in glycerin and drawing water under the cover slip while watching the preparation. No change could be seen in the grains until they were struck by the oncoming water, and then the change was very rapid and its successive stages could not be distinguished. Experiments with various solutions of glycerin and water were also unsuccessful.

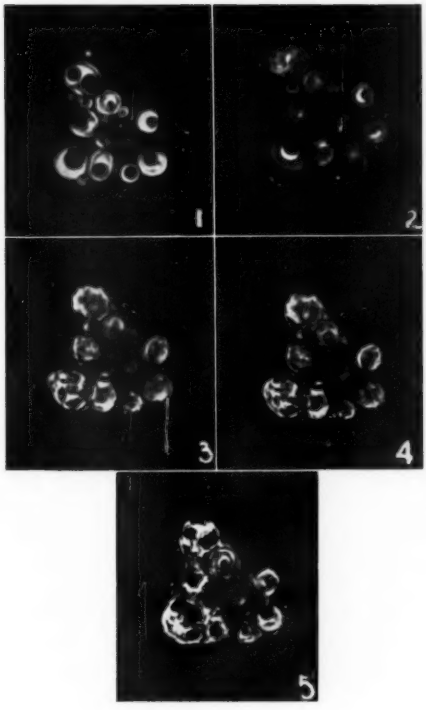
The procedure finally followed was as follows. Through a thin microscope slide (dimensions 2 in. × 3 in.) two holes were ground, each about one-half inch in diameter, and were connected by grinding a channel from one to the other. By cementing a large cover slip to the underside of the slide, two connected chambers (or, when the channel was wide, one much elongated chamber) with an optically plane floor were produced. The material for observation was prepared by dissecting the embryo from a seed and crushing it between cover slips. Care was necessary to avoid crushing the grains themselves, but it was possible to obtain a scattering of whole grains and small groups of grains adhering to the cover slip. These were immersed in a drop of glycerin, and the cover slip was placed on the prepared slide so that the hanging drop in which the grains were immersed fused with another drop

that had been placed on the floor of one of the chambers. The other chamber contained a large drop of water, which slowly evaporated and diffused over to be absorbed by the glycerin. With the cover sealed to the slide with vaseline, drying up was prevented, and the preparation could be observed over a period of hours with a slowly increasing concentration of water about the grains. The conditions differed from those in the germinating seed in two respects. The action was slow and gradual, and there was no confinement or distortion of the grains by external pressure.

Stages in the reaction of the grains are illustrated in the plate, which shows successive photographs of the same group at the same magnification. Figure 1 was photographed 28 minutes after the application of the glycerin. The grains still had their original appearance, similar to that of grains mounted in oil with no access to water. Two hours and 5 minutes later, as shown in Fig. 2, they had changed considerably. They had swollen, and while the outlines of the granules were still indicated in some cases, the clearly defined difference in appearance between granules and ground substance was no longer evident. Figure 3 is from a photograph made 1 hour and 4 minutes after Fig. 2. There was now slightly more swelling than shown in Fig. 2, and the contents of the grains appeared still more disorganized, no indication remaining of the shape of the original granules. Well before this the grains had reached a stage in which, if examined without the aid of the phase microscope, they appeared practically homogeneous. When Fig. 4 was taken, 2 hours later, the grains still appeared disorganized, but there was an increase in contrast between light and dark parts. During the 2 hours the size of the grains had not increased. Figure 5 shows the group as it appeared next day, between 23 and 24 hours after the mount was made. There had been some apparent shrinkage in the grains, and the light and dark parts were now more clearly outlined than at any former time.

In the light of many observations, obscure parts of the process have become clarified. The substance of the aleurone grain absorbs water and swells, and the refractive indices of granules and ground substance approach each other during the process. Later, swelling of the ground substance reaches a maximum, but the material of the granules continues to absorb water, becomes liquid, and diffuses out into the surrounding medium. Under treatment causing the changes to develop rapidly, one can often see the ground substance surrounded by a much larger vesicle of the colloidal suspension that has been extruded from the dark granules and has not yet had time to diffuse away. The ground substance itself has become soft and jelly-like. Its form has become distorted to some extent, as shown in the photographs, even when care has been taken to avoid distorting influences as much as possible. With more rapid expansion, especially when accompanied by the turbulence of flowing liquid in the mount and compression by the cover slip, this substance often becomes broken, each piece rounding up into a small, bright globule. Such a transformation invariably takes place in the seed at one stage in its germination. I have not been successful in sectioning or staining the tissues at this stage without disarranging the very mobile material,

PLATE I



FIGS. 1 to 5. Successive photographs (1050 diameters) of a group of swelling aleurone grains mounted in glycerin which is slowly absorbing water vapor.

FIG. 1. Grains freshly mounted. Dark granules clearly visible.

FIG. 2. Photographed 125 minutes later. Grains somewhat swollen. Granules and ground substance not clearly distinguishable.

FIG. 3. Appearance of grains 64 minutes after Fig. 2. Grains more swollen and more disorganized than in Fig. 2.

FIG. 4. Appearance of grains 120 minutes after Fig. 3. No increase in size of visible portion. The liquefied content of the dark granules is diffusing out, resulting in more contrast in the photograph.

FIG. 5. More than 23 hours after the mount was made. The ground substance still appears white, but slightly shrunken. The dark parts are darker than in the earlier stages, the content of the granules having been replaced by the mounting liquid.

but one can observe the various stages in the cells of whole embryos, by the use of an oil immersion objective. With the grains confined within the cells of the germinating seed, the liquefied substance of the granules runs together within the grain before escaping from it. At one stage each grain is seen, much enlarged, and composed of a liquid central mass (the dark granules having run together) surrounded by a layer of ground substance, more or less broken up. Later, the liquid portions of adjacent grains run together, the pockets of liquid becoming progressively larger and fewer, but each surrounded by the globules of ground substance of its component grains. Still later, each cell has one large vacuole and a thin layer of cytoplasm richly supplied with the bright globules.

Swelling of Cells of the Embryo

By trial and error it was established that the cells of germinating embryos could best be observed in whole embryos dissected out without injury. In earlier work each embryo was split in two with a sharp razor, and the cells at or near the cut surface were examined. By this method it was established that the epidermal and cortical cells were essentially similar morphologically, though the aleurone grains were more clearly visible in the cortex. Moreover, the process of swelling in the inner cells was somewhat more rapid than in those of the epidermis, but otherwise the reaction was similar in the two regions. In the case of swollen, partially germinated seeds it was not possible to make the cut without some distortion and breaking up of the softened aleurone grains, and for this reason the observations now to be described were all made on epidermal cells of intact embryos. By grinding a hole through a No. 1 glass cover slip and cementing the slip to a microscope slide, a well was produced, deep enough to prevent crushing of the embryo, and sufficiently shallow for convenient observation of the preparation under an oil immersion objective. The embryos to be studied were mounted in paraffin oil to avoid any gain or loss of water during observation.

The structural changes occurring in the cells under various sets of conditions will be more understandable if a general account of their process of swelling and vacuolation is first undertaken. Dangeard's idea of the aleurone grains was that they were merely vacuoles whose water had been extracted and would be regained at the first opportunity. The swelling and liquefaction of the dark granules in the grains of *Typha* undoubtedly corresponds to what he observed. In this material, however, the central vacuole does not originate from the swollen grains, although their liquid parts undoubtedly contribute to it. It is commonly to be seen in the cell while the grains are still intact, and before they have reached the stage where their liquid condition is apparent. It first appears as a minute cleft among the grains, clearly distinguishable from the protoplasmic matrix in which they are embedded. The cleft grows into a small vacuole which is star-shaped because the surrounding grains, still preserving their shape, press into it from all sides. At this stage the vacuole appears dark under the microscope. Later its size increases to the point at which the dark appearance is lost. Even at this stage the aleurone

grains are still intact and pressing into the vacuole. Later their liquefied portions run together, fusing with each other and with it.

The breaking of the seed coat is apparently a critical point in the germination of these seeds. Unless the cap has been removed artificially, germination depends on the generation by the swelling embryo of sufficient pressure to break the ring about the cap. In the mature seed there is a small space between the cap and the radicle, and swelling sufficient to fill this space must take place before pressure is exerted. Under any of the conditions provided in these experiments, enlargement of vacuoles and aleurone grains, combined with whatever swelling takes place in other colloidal parts, is sufficient to fill the space. There is evidence the energy that breaks the coat is supplied jointly by vacuolation and swelling of the grains, and that, in germinable seeds, the effect of the environment on these two processes is responsible for success or failure in germination.

Effect of Environment on the Swelling

Light

When seeds are placed under ideal conditions for germination, in distilled water at 30° C with light, the swelling of aleurone grains is more striking than the production of central vacuoles. Before the coat breaks, all the grains swell, as a rule, until they are pressing together and have lost all appearance of dark granules. They cannot, in the whole embryo, be observed to advantage under the phase microscope, but have apparently reached at least the stage shown in Fig. 2. There is still no appearance of liquid content in the grains, but they have a slightly pebbled appearance on their surface, probably due to the beginning of granulation of the matrix. Of 45 embryos examined at this stage, after periods of from $4\frac{1}{2}$ hours to somewhat more than a day under the germinating conditions, 36 had the grains as described above from end to end of the embryo. Of the rest, one had all its grains less swollen than this, one gave the typical appearance toward the chalazal end but had grains less swollen toward the cap or radicle end, and seven had the grains near the chalazal end more swollen and appearing liquid. The variations seem to be due to peculiarities of individual seeds, or at least to some cause other than the time spent in the germinator. Of the two seeds with grains less swollen than the rest, for example, one had been in for $4\frac{1}{2}$ hours, and the other for $24\frac{1}{2}$ hours.

These seeds had also developed central vacuoles with the grains pressing into them. Of the 45 seeds, 37 had so-called dark vacuoles (too small to have a light appearance) near the chalazal end, two had similar ones from end to end, and the six with liquid grains had the star-shaped dark vacuoles where the liquid grains were absent.

It is to be noted that wherever one part of a seed has swollen more than another, the swollen part is near the chalazal end. This is invariably true in seeds with unbroken coats, indicating the point at which water enters the seed.

Once the cap has been forced from the seed and pressure on the embryo is released, further absorption of water is very rapid. Seeds were left in the germinator, under water, in light, at 30° C until a number had germinated, and the remainder were watched. When a seed was seen to have forced off its cap, it was removed promptly, and the embryo was dissected out and mounted in oil as rapidly as possible. Of 36 seeds treated in this way, 22 had the aleurone grains clearly liquefied for distances of from 1/4 to 5/7 of the length of the embryo from the chalazal end, five had all the grains liquefied, and nine had the grains liquid at both ends but not at the center. In many cases the liquid of adjacent grains had run together, giving the appearance of fewer, larger grains with liquid centers surrounded by the highly refractive granules of the ground substance. Where the running together stage had been reached, the central vacuoles could not be distinguished. In other parts the grains and vacuoles were as in the soaked, but still unbroken, seeds. If left in water after breaking out, the embryos elongated with great rapidity, and the cells soon had one large vacuole each, composed of the original central one and the liquid part of the grains, surrounded by a peripheral layer of protoplasm crowded with granules of the ground substance.

Darkness

Forty-six seeds were examined after from 1 to 6 days in the germinating tank in complete darkness. Growth of the central vacuoles corresponded remarkably well with that in light. Forty embryos had small vacuoles extending for various distances from the chalazal end, three had them extending from end to end, and in three they were not evident. The aleurone grains showed a different picture. Of the 45 embryos examined after immersion in light and before they had broken their coats, 36 had the grains swollen until they pressed together strongly throughout. In darkness this happened only in eight of the 46 examined. In 21 others they were pressing together to some extent, but visibly less so at the radicle than at the chalazal end, and 12 more had them slightly swollen, more so at the chalazal end, but pressing together little or not at all. There were four seeds in this experiment that, like the seven among those in light, had a few cells near the tip with grains appearing liquid.

Quite definitely, one difference between the reaction of *Typha* seeds in light and in darkness is that the aleurone grains of the latter have less swelling power. The experimental results cannot be attributed to insufficient time. After 6 and 7 days in darkness, the swelling was less than after 4½ hours in light, and in another experiment seeds were left in the germinator in darkness for some months and still had grains no more swollen than those left for 1 or 2 days.

Blue Light Filters

In light filtered through blue cellophane, of 39 seeds left in water at 30° C for 1 to 6 days, eight had some grains appearing liquid near the chalazal end and the rest pressing together, 31 had all the grains pressing together, but in 14 of these the flattening by mutual contact was visibly less toward the radicle

end, and in 17 they were pressing only slightly throughout. Thirty-four of these seeds had small central vacuoles toward the chalazal end only, and five had none visible. The effect of the blue filter was thus similar to that of darkness insofar as swelling of grains was concerned, and like darkness it brought about little or no modification in the production of central vacuoles. In each of the three experiments so far described, these vacuoles were small, and their average range was from 5/16 to 3/7 of the length of the embryo from the chalazal end.

Air

As recorded earlier, germination in air presented some complexity. It will be recalled that under clear light and at favorable constant temperature, some samples gave a much reduced percentage of germination in air and others did not. In seeds incubated in air, it was more difficult to see what was happening in the cells, and this proved to be due to the increased number and size of the central vacuoles, which rendered the embryos more opaque. Of the 32 embryos examined after treatment in air with clear light, all had central vacuoles clearly visible from end to end except in a few cases where liquefied grains obscured some of them. All of these embryos were dissected out and mounted as soon as possible after, not before, they had pushed the caps off. Of the 32 embryos, 24 had their aleurone grains more or less swollen, but showing no signs of liquefaction, two contained some grains about which there was doubt, and six had some liquid grains. These are to be compared with the 36 embryos described on page 731, treated in the same way except that they were under water, all of which had liquefied grains near the chalazal end, while nine of them showed such at both ends, and five had them extending from end to end. In the present experiment, by the way, six is probably too high a number for embryos having some grains appearing liquid at the time of breaking of the coats. There were some indications that after being mounted in oil, some of the grains, with the pressure of the seed coats removed, might have been absorbing water from the comparatively large central vacuoles. As a result, care was taken in the last 16 cases to examine the embryos promptly after mounting, and as rapidly as possible, and in these embryos no liquefied grains were present. It was evident from these results that a free supply of air during the period before the coat was broken discouraged swelling of the aleurone grains, but encouraged the formation and enlargement of central vacuoles.

This conclusion as to the influence of oxygen supply was amply confirmed by the behavior in blue light and in darkness. Of 22 seeds left from 1 to 12 days in air and blue light, and 44 in air and darkness, only one had some grains, near the chalazal end, with a liquid appearance, and in only eight were they noted as pressing together. The central vacuoles in these resembled those developed in clear light and air.

Alternating Temperatures

Seeds from the lot that gave sporadic but increased germination in darkness when temperatures were alternated were placed under water in darkness with

the alternating temperatures. Embryos from 27 of these seeds were examined after various lengths of treatment between 22 and 72 hours, and the results were striking. The aleurone grains behaved as they did in darkness at constant temperature, except that on the whole the swelling was noticeably less rapid, due in part perhaps to slow diffusion during the lower temperature periods. The central vacuoles, on the other hand, were larger and more plentiful than when constant temperatures were employed, and in 23 of the 27 embryos they were clearly present from end to end. Without doubt the favorable effect of the alternating temperatures on germination was due to vacuole formation.

Five additional seeds were examined, but are not included in the above summary because of irregularities in the dissection procedure (exposure to direct sunlight, or brief lack of contact with the moist blotter) which may have led to the loss of some water before mounting. They are of interest, however. Their aleurone grains appeared to be little swollen, and the dark granules were clearly visible. Even if this is due to loss of water during dissection, it indicates at least that the granules were, before loss of the water, still separate entities, and had not liquefied to the extent of running together. These seeds had all been in the germinator for between 2½ and 3 days.

Effect of Removal of Caps

Seeds with caps artificially removed were incubated at 30° C in darkness and in light, in air and under water, and after various lengths of time their embryos were examined. Records of the swelling of aleurone grains under these conditions are shown in Table VII. In this case, since the caps had been removed, there was not, as in the seeds already reported on, a critical point

TABLE VII

Swelling of aleurone grains at 30° C. Seeds with caps removed

Environment, and condition of grains	Duration of treatment in minutes to produce the condition
Light, water	
Grains pressing together throughout	35, 45, 55, 55, 65, 70, 70, 70, 75, 85, 100, 100, 115
Grains at least touching	75, 85
Some grains not touching	35, 35, 40, 40
Dark, water	
Grains pressing together throughout	40, 63, 70, 70, 125, 145, 185, 225
Grains at least touching	75, 75, 95, 165
Some grains not touching	35, 35, 38, 40, 43, 45, 53, 60, 60
Light, air	
Grains pressing together throughout	55, 57, 68, 75, 75, 83, 90, 90, 93, 95
Grains at least touching	65, 73
Some grains not touching	35, 37, 40, 42, 45, 45, 50, 67
Dark, air	
Some grains liquid, rest pressing	170, 220, 225, 230
Grains pressing together throughout	100, 115, 130, 135, 135, 140, 145, 155, 165
Grains at least touching	180
Some grains not touching	75, 80, 125

where swelling stopped or slowed because of pressure of the seed coat, and therefore the report necessarily takes a different form. For purposes of comparison, the seeds of each experiment are arranged in groups depending on the amount of swelling of the grains. Each number in the table records the number of minutes between the time when an individual seed was placed in the germinator and the time when its aleurone grains were examined. The latter part of this interval, while the seeds were being mounted for examination, was in each case at room temperature, somewhat lower than the 30° C of the germinator. One point shown in this table is that, when relieved of confinement by the seed coat, the aleurone grains swell rather promptly under all the environmental conditions of the experiments. The longest time allowed was less than 4 hours, and well within that time many of the seeds under all the sets of conditions had their grains pressing together throughout, and some had liquefied grains, even when incubated in darkness and air. It is also indicated that the swelling is more prompt in light than in darkness. In the immersed seeds the swelling is more prompt than in those in air, but one cannot be sure that this is not partly due to a greater availability of water to seeds surrounded by the liquid medium. The effect of oxygen on the growth of vacuoles is also obscured by this same factor.

In the seeds immersed in water the grains were often more swollen at the two ends than near the center, and the same was true of the central vacuoles. This is in sharp contrast to what takes place when the intact cap is still on the seed. Even with the caps removed, however, the less swollen part, when still present, was nearer to the radicle than to the chalazal end. Apparently water is still absorbed more rapidly through the wick-like tissue of the chalaza. In seeds absorbing water from moist filter paper the picture is more complicated and less regular, apparently depending to some extent on which end of the seed is in closer contact with the moist paper.

Respiration of Germinating Seeds

The pronounced effect of lowered oxygen pressure on germination and on vacuolation and the swelling of aleurone grains suggested an investigation of respiration. The Warburg apparatus was used, and records were made of the respiration of the germinating seeds at various temperatures, in air and in air diluted with nitrogen to the point at which oxygen concentration at the beginning of the tests was about 2%.

At the beginning of each experiment there was a period when a factor other than respiration was active, bringing about an increase of volume in the contents of the experimental flasks and manometers. In many cases there was at first an increase in volume, even in flasks containing a solution of caustic potash for the rapid absorption of the carbon dioxide produced. The net result was that the apparent respiratory quotient during this period was very considerably higher than the real. The duration of the period was clearly indicated when frequent readings were made and successive apparent respiratory quotients plotted on a curve. The initial hump in this curve, due to the extraneous factor, ended rather sharply, and the beginning of the

TABLE VIII

Respiration and respiratory quotient of germinating seeds
(Figures for oxygen and carbon dioxide represent amounts per g per hr)

	In air			In 2% oxygen		
Temp.	Oxygen used, cu. mm	Carbon dioxide produced, cu. mm	R.Q.	Oxygen used, cu. mm	Carbon dioxide produced, cu. mm	R.Q.
10° C	18.539	13.704	0.73	10.499	10.250	0.98
15° C	25.723	21.410	0.83	16.351	19.437	1.19
20° C	54.551	44.845	0.82	17.822	30.738	1.72
30° C	190.027	173.400	0.91	19.477	82.018	4.21

flattened part of the curve was taken as indicating the time when the Warburg readings became indicative of the actual gas exchange. At 30° C this point was reached in from 3 to 4 hours after the beginning of the experiment, at 20° C 6 hours or more were required, at 15° C about 8 hours, and at 10° C 10 hours. The results shown in Table VIII are from readings taken after this initial period was over.

Two points are clearly indicated:

(1) The rate of respiration is greater in air than at the lower concentration of oxygen, and it increases with rise in temperature up to 30° C, the rate of increase being greater in air, and becoming notably greater as the optimum temperature for germination is approached.

(2) The respiratory quotient also increases with rise in temperature, but in air the increase is small, and the quotient is below 1 even at 30° C. With the low concentration of oxygen, on the other hand, it is slightly above 1 at 15° C and rises rapidly as the optimum temperature is approached, reaching 4.21 at 30° C. The R. Q. of 4.21 is the average for the 7 hours immediately following the initial period of swelling mentioned above. During the time of the experiment, the R. Q. was slowly rising, due, presumably, to the gradual using up of oxygen from the small amount in the flask.

Discussion

There is, in the reactions of these seeds, a basis for progress toward a better understanding of the germination process. In them, as in many others, the breaking of the coat is a critical development. Failure to germinate was usually due to inability to rupture the ring of tissue uniting the cap that covers the radicle with the remainder of the seed coat. In building up the necessary pressure, two processes that could be followed microscopically were active, namely the swelling of aleurone grains by the absorption of water, and the growth of central vacuoles in the cells.

Under all the conditions tried, some swelling of the grains took place, and if unconfined they continued to absorb water until the substance of their dark granules became liquid and freed itself from the softened matrix in which the granules were embedded. In the presence of light of suitable color combined with low oxygen pressure, this swelling was much more rapid than

in darkness, or in blue light, or with oxygen at atmospheric pressure. Under the favorable conditions it was also markedly more vigorous, and proceeded notwithstanding the confining pressure of intact seed coats, until the coats were broken. In darkness or under the blue light filter, the increasing pressure of the coats was sufficient to bring the swelling to an end before the coats were broken. A similar effect resulted from the higher oxygen pressure, though a percentage of the seeds, higher in some samples than in others, did break out from the coats when incubated in air, provided light of longer wave lengths was supplied.

The contemporaneous process of swelling by vacuolation was apparently independent of light or darkness, but was markedly stimulated by the higher oxygen pressures that depressed the swelling of the grains, thus accounting for the germination in air of some seeds.

Under the lower pressures of oxygen, respiration was comparatively slow, and the respiratory quotient was high, especially at the optimum temperature for germination. In air, on the other hand, the respiratory quotient was less than one, and respiration was more than twice as rapid.

It appears that when the aleurone grains swell, a certain amount of water is absorbed and held strongly by their colloidal material. Additional swelling takes place if inhibiting effects such as seed-coat pressure are removed, but the additional water is attracted and bound less strongly. The contribution of the aleurone material to breaking the seed coat would under these circumstances depend on the balance between the attractive power of the colloid and the constricting pressure of the coat. The reaction of the aleurone grains throughout the investigation is explainable on the hypothesis that the amount of water that can be strongly held by the colloids is increased by the action of the longer wave lengths of visible light, and by the presence of the products of anaerobic respiration. Perhaps the initial swelling that was evident in the Warburg readings was due to reorientation of the adsorbed water molecules on the colloidal surfaces.

The initiation and growth of the central vacuoles takes place under all conditions investigated. Under low concentrations of oxygen the vacuoles are small, soon reaching a stage where expansion ceases under pressure from the seed coat. When the seeds are incubated in air and light, the more vigorous growth of these vacuoles is apparent, and in a certain percentage of seeds is sufficient to compensate for the lesser amount of strongly held water in the aleurone grains, and so to bring about rupture of the coats and germination. In air and darkness the vacuoles expand much as they do in air and light, but with the drastically reduced swelling of aleurone grains the coat is not broken. Apparently the tendency for water to enter the vacuoles, gradually decreasing as the cell sap absorbs more water, is not sufficient to break the coat.

At low light intensity, the use of alternating temperatures improves the germination percentage. In some samples of seed sporadic germination is induced by this treatment even in complete darkness. To this latter group belong the seeds that germinate well in light and air, which suggests that the

vigorous growth of vacuoles may play a part, and microscopic observation bears this out. In darkness the aleurone grains swell rather more slowly with alternating temperatures than without, but the opposite is true in the case of the vacuoles. The stimulating effect of alternating temperatures on growth of vacuoles is explainable on the basis of respiration results at the various temperatures. At low temperatures the respiratory quotient and the rate of respiration are both greatly reduced, allowing, if the time spent at low temperature is sufficiently long, for getting rid of respiration products, and development within the seed of conditions that on the resumption of higher temperatures will bring about more rapid respiration and more vigorous growth of the vacuoles.

Conclusions

1. The optimum temperature for the germination of these seeds is close to 30° C. Above this temperature the germination percentage decreases. At temperatures below 25° C, both percentage and rapidity of germination decrease rapidly as the temperature is lowered.

2. Low oxygen pressure, whether brought about by immersion in water or otherwise, gives consistently better germination with some samples. Others germinate equally well in air.

3. Without light there is little or no germination at optimum constant temperature. At low light intensities the seeds are sensitive to very small differences.

4. At low light intensity, germination is consistently improved by rather wide alternations of temperature.

5. With certain samples, alternating temperatures fail completely to overcome the inhibiting effects of complete darkness. In others the alternations bring about sporadic germination with all light excluded. This second result is obtained with seeds that germinate well in air, without reduced oxygen concentration.

6. The inhibiting effect of a blue light filter is very little affected by alternating temperatures.

7. Removal of caps from the seeds obviates the necessity for light in germination, and pricking of the coat has a similar, but more limited, effect. The advantageous effect of cap removal is less in blue light than in darkness.

8. Parenchyma cells of the embryo are richly supplied with aleurone grains with a ground substance that appears white, and contains numbers of dark granules.

9. When allowed to do so, these grains absorb water and swell, the dark granules becoming liquid while the ground substance becomes plastic so that it is easily distorted and broken up.

10. While the grains are swelling during germination, central vacuoles are forming in the cells, appearing first as dark clefts between grains, later as star-shaped structures owing to the pressure of the surrounding grains, and still later as normal vacuoles, after the grains have lost their liquefied portion and become completely plastic.

11. The swelling of the aleurone grains is much more rapid and vigorous in white or yellow light than in darkness or in blue light. It is also aided by immersion of the seeds in water and thus lowering the oxygen supply.

12. Normal vacuolation, as distinct from liquefaction in the aleurone grains, is enhanced by higher oxygen pressure, and impeded by immersion of the seeds in water.

13. Alternation of temperatures has no favorable effect on the swelling of the aleurone grains, but does favor vacuolation.

14. The rate of respiration of germinating seeds is greater in air than with the reduced oxygen pressure that favors germination. With rise in temperature to the optimum for germination the rate of respiration increases, the rate of increase being much greater as the optimum temperature is approached.

15. The respiratory quotient at 30° C was found to be 0.91 in air, and 4.21 in 2% oxygen, which gave best germination. With lower temperatures the quotient is lower.

16. The germination behavior of these seeds is explainable on the premise that the amount of water that can be strongly attracted and bound by the colloidal proteins of the aleurone grains is increased by the action of certain wave lengths of light and by the presence of products of anaerobic respiration. Vigorous aerobic respiration, on the other hand, favors vacuolation, and in seeds that vacuolate strongly may to some extent compensate for weaker swelling power in the grains. The wave lengths passing our blue filter have an inhibiting effect on which this investigation throws no light, but which is more difficult to overcome than that of darkness. The stimulation of vacuolation by alternating temperatures is reasonable in the light of their effect on respiration.

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NOTES

GERMINATION BEHAVIOR OF AEGILOPS KOTSCHYI BOISS.

YOAV WAISEL AND YEHUDIT ADLER

Dormancy in recently matured grains in the Gramineae was established by many authors (2, 3, 4, 6, etc.), although the factors causing it remain generally obscure. In the course of some germination experiments, a correlation was found between the duration of dormancy and the temperature required for germination of *Aegilops kotschyi*, suggesting the possible mechanism by which dormancy is caused. Therefore, a more detailed investigation into the factors controlling germination of this grass species was undertaken.

Spikes of *Aegilops kotschyi* collected in the Northern Negev of Israel in the springs of 1957 and 1958 were used in these experiments, and examined both when they were recently matured and after they had been stored up to 1 year. Germination of the following constituents of the dispersal unit was investigated: (*a*) entire spikelets, (*b*) "florets", i.e. caryopses within the pales, (*c*) naked caryopses, (*d*) detached caryopses reinserted within the pales, (*e*) spikelets pricked with a pin through the envelopes.

"Florets" and caryopses from the lower position in the spikelets (lower unit) were germinated separately from the upper ones (upper unit). Tests were carried out simultaneously at a series of temperatures ranging from 5° C to 30° C.

Germination of recently matured spikelets and of their parts was found to decrease with increase in temperature; for spikelets, highest per cent of germination (about 70%) was obtained at 5° C, while at 30° C no germination took place. At this temperature only naked lower caryopses germinated (about 57%). Generally, germination was positively correlated with the gradual removal of the floral envelopes, both in recently matured and in stored material; e.g. percentage germination in recently matured material, at 20° C, was 3.9, 47.5, and 77.3 for entire spikelets, for lower "florets", and for naked lower caryopses respectively. At 5° C, percentage germination obtained for the same parts was 69.9, 85.8, and 97.3 respectively.

An increase in percentage germination was found in all temperatures tested, after storage of the germination units.

The above results suggested that an inhibitory substance, whose activity increases with temperature, is present in the glumes, in the pales, and probably also in the seed coat (5). Accordingly, the effect of extracts of glumes and pales of recently matured spikelets was tested on germination of stored caryopses of *Aegilops kotschyi*, as well as on lettuce seeds. The results showed that the extracts were in fact inhibitory to germination. Furthermore, the inhibitory activity of the extracts was found to decrease with increased duration of storage of the spikelets; e.g. percentage germination of

lettuce seeds in extracts of recently matured chaff and of material stored for 4 months was 16.3 and 31.0 respectively, as against 62.3% in tap water. This suggests that the inhibitor gradually decomposes during prolonged storage.

In recently matured material, differences were found between germination of the lower and the upper "florets" and caryopses, as manifested by higher germination percentages of the lower ones as against the upper ones. These results possibly imply a greater sensitivity of the upper caryopses to the presence of the inhibitory substance. No such differences were found after 1 year's storage, when both groups of caryopses germinated fully.

In order to find out the possible effect on germination of mechanical damage to the envelopes, detached caryopses were reinserted within the pales and tested along with pricked spikelets. Both treatments favorably affected the germination of recently matured spikelets or "florets"; e.g. per cent germination of pricked spikelets (material stored for 4 months and germinated at 30° C) was 38.3 as against 20.4 for entire spikelets and 90.4 for naked lower caryopses. Results show that due to the presence of the floral envelopes it was the inhibitory substance and not the injured seed coat dominating the control of germination.

This inhibitory substance is probably the factor responsible for the prevention of germination in *Aegilops kotschyi* during the dry and hot summer months. Since this plant is a desert winter annual, such a mechanism for control of germination seems to be of ecological importance (1).

Five other grass species, *Aegilops longissima* Schw. et Muschl., *A. ovata* L., *Bromus scoparius* L., *Hordeum murinum* L., and *Hyparrhenia hirta* Stapf, tested on these lines, revealed a similar behavior in germination, thus suggesting that germination control of this nature might be a general phenomenon in the Gramineae.

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